A Dissertation for the Degree of Doctor of Philosophy in Pharmacy

Synthesis and Biological Evaluation of Carbocyclic Nucleosides

카보사이클릭 뉴클레오사이드의 합성과 생물학적 활성 검증

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SYNTHESIS AND BIOLOGICAL EVAULATION OF CARBOCYCLIC NUCLEOSIDES

by

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UNDER THE GUIDANCE OF

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Abstract

The sugar core of a nucleoside is known to exist in a rapid equilibrium of the two extreme forms, namely the north and the south conformations. Conformation restricted modified nucleosides have been developed as a tool to better understand the physicochemical nature of diverse biological interactions, and the information revealed during the process provided enormous possibility of these unnatural nucleosides for potential therapeutic use. The bicyclo[3.1.0]hexane, structure with a carbocyclic core, serves as a privileged template to lock a given nucleoside in an either conformation, where its uses are actively being reported to date. However, synthetic difficulty of these compounds, the south especially, prevents such valuable template from being developed any further. In this study, an efficient route to access south conformation restricted nucleosides built on a bicyclo[3.1.0]hexane has been explored. As a result, the synthesis of south methanocarba uridine was achieved in 7% overall yield in 15 steps from easily accessible D-ribose. The current study is expected to serve as a valuable resource for further development of conformation restricted nucleosides. In a continuing effort in discovering the role conformations in nucleosides, a precursor of south methanocarba cytidine phosphonamidite was prepared to observe the effect of conformation restricted nucleosides in leadzyme self-cleavage activity.

An alternative route to access MLN4924, a NEDD8-activating enzyme (NAE) inhibitor which is in phase 1 clinical trial as an anticancer agent has been explored for further structure-activity relationship studies. The synthetic route proposed in this study includes regioselective α-alkoxy removal and stereoselective reduction as key steps, which successfully provided MLN4924 in an overall yield of 13% in 15 steps. Furthermore, in continuation to the structure-activity relationship study of MLN4924, fluorinated analogues were designed based on bioisosteric rationale, and their syntheses were achieved employing stereoselective reduction, regioselective isopropylidene cleavage and DAST fluorination as key steps.

Keywords: carbocyclic nucleosides, conformation restricted nucleosides, MLN4924, fluorinated nucleosides, bioisosteres

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Contents

Part I.

Synthesis of South Conformation-Locked Nucleosides

1. Introduction

1.1 Introduction to Sugar Conformation

The discovery of the double helix structure of DNA in 1953,[1] has played a crucial role in the field of nucleic acids research, providing grounds for better understanding fundamental aspects of human nature.[2] While intense researches in the past years revealed the immense possibility of nucleic acids as therapeutic tool,[3] what has relatively not been understood was why DNA exist in different shapes, namely A, B, and Z-forms, and what causes them to exist as they are. Development of X-ray crystallography and spectroscopic technologies have disclosed that these forms differ in their sugar conformations, with C2'-*endo* conformation predominantly found in B-form DNAs while C3'-*endo* in A-form helices.[4] Since then, sugar conformation and its influence on biological interactions has been an interesting topic for investigation.

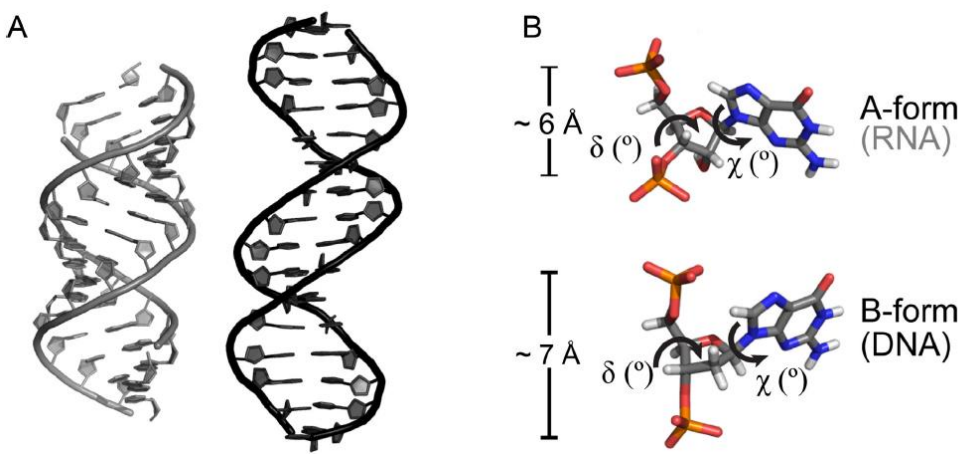


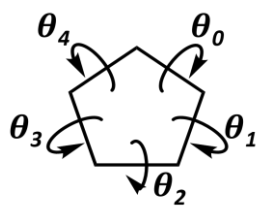
Figure 1. (A) The structures of A-form and B-form nucleic acids and their (B) respective sugar conformations. Figure redrawn from the work of Anosova I., *et. al*.[5]

In order to define what sugar conformation is, an in-depth understanding of physicochemical properties of a 5-membered ring must be preceded as it constitutes the core of nucleosides. The molecular structure of a simplest 5-membered ring, the cyclopentane has been explored in detail suggesting the

structure is indefinite, transforming its shape continuously, and the relationship of the freely rotating conformation can be presented as a term of pseudorotaion.[6] However, when one or more substituent is present, the potential energy dramatically changes and causes restriction to freely rotating puckering by the influence of strain energies.[7] The concept of the pseudorotation was then expanded to the field of nucleoside and nucleotide chemistry, corroborating theoretical, computational understandings of sugar puckering with the observed structural evidences obtained by X-ray crystallography.[8]

1.2 The Pseudoroational Cycle

Because the torsion angles θ_0 to θ_4, presented in Figure 2., are interdependent to each other, the pseudorotation pathway of a cyclopentane can be described as a function of cosine,[9] and the pseudorotational angle at the lowest possible energy, derived by the formula presented in Figure 2,[9] would fall in between a one full pseudorotational cycle 0 to 360°.[8]



$$\tan P = \frac{(\theta_2 + \theta_4) - (\theta_1 + \theta_3)}{2\theta_0(\sin 36 + \sin 72)}$$

Figure 2. Graphical representations of torsion angles θ_0 to θ_4, and their relationship presented in terms of pseudorotational angle *P*.

Applying the same concept to sugar conformation, the pseudorotational pathway of a furanose ring can be presented by a pseudorotational cycle shown in Figure 3, where *T* represents twist conformation and *E* represent envelope. Pseudorotational angle *P* at 0° (*P* = 0°) is defined an absolute north conformation while *P* = 180° corresponds to an absolute south. The number on the upper side of the letter represents the atom located above the

given plane, whereas the number below represents the atom located below. An absolute north conformation for instance with P value of 0°, $^{3}T_{2}$ represents a twist conformation with a symmetrical C3 above (C3'-*endo*), and C2 (C2'-*exo*) below the plane.[8] According to the work of Sundaralingam and co-workers, majority of β-purine glycosides fall in the range in between P = 2 to 14° (north type) and P = 139 to 174° (south type), whereas β-pyrimidine glycosides fall in the range of P = 3 to 15° (north type) and P = 161 to 175° (south type).[8]

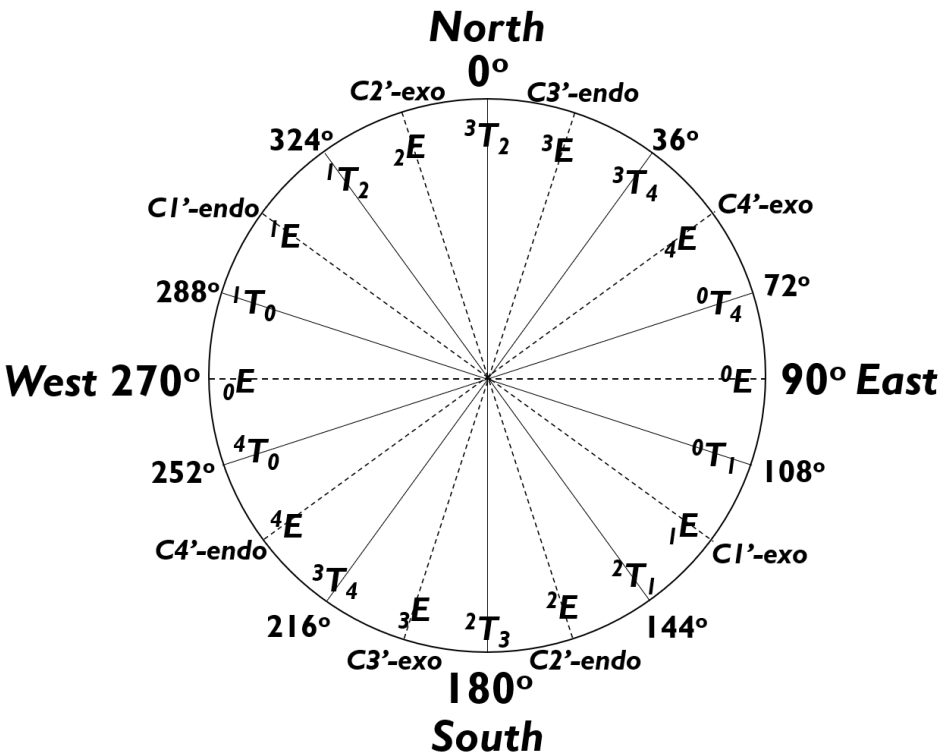


Figure 3. Pseudorotational cycle 0° < P < 360°. *T* represents twist conformation and *E* represents envelope. The absolute north corresponds to P = 0° and absolute south corresponds to 180°.

While the pseudorotational angle may accurately represent the structure of a given nucleos(t)ides in a solid state, which is determined by anomeric, gauche effects[4a] and crystal packing forces,[4a,10] in solution state the conformation is known to exist as a rapid equilibrium in between the north

and south conformations.[4a,8] Since only of these conformations are expected to be optimal for effective binding with the target enzyme, any conformational studies based either on a solid state analysis or solution state would be inaccurate.[11] Thus, nucleos(t)ides with a fixed conformation was desirable for further and accurate study on the role of conformation in biological interactions.

1.3 The Bicyclo[3.1.0]hexane as a Conformation-Restricted Nucleoside Template

The X-ray structure of Neplanocin C, a naturally occurring nucleoside analogue shown in Figure 4, has shown that having a bicyclic core restricts the conformational equilibrium and locks the 5-membered core into a fixed conformation, a typical north conformation, which was consistent both in solid state X-ray analysis and solution state NMR studies.[12]

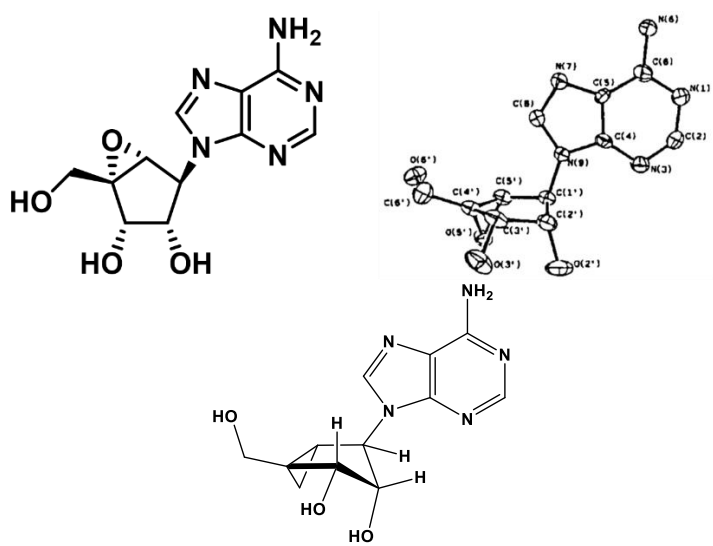


Figure 4. Structure of Neplanocin C (top-left) and its crystal structure (top-right). A more stable carbocyclic nucleoside built on a bicyclo[3.1.0]hexane was developed as a north-locked nucleoside template (bottom).

Because epoxides are chemically labile, a more stable cyclopropyl-fused, as a replacement of the epoxide ring, template has been developed. This

bicyclo[3.1.0]hexane template indeed displayed a rigid scaffold exhibiting a typical north geometry with better stability.[13] Further examples confirmed the validity of the bicyclo[3.1.0]hexane as conformation-restricted template.[14] The south template was also validated based on the location of the cyclopropyl being installed.[14b] (Figure 5.)

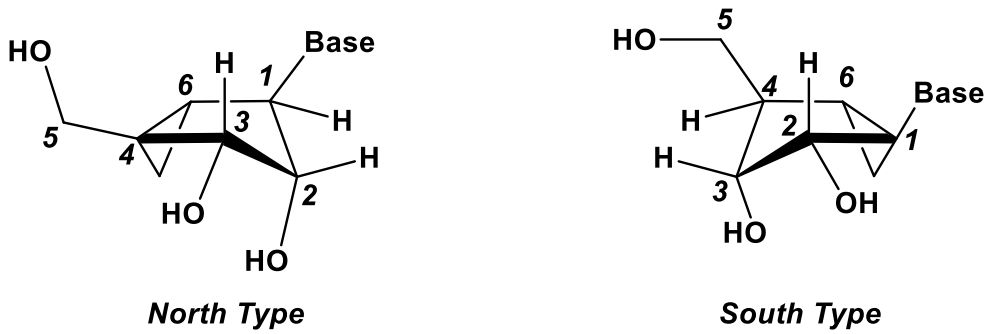


Figure 5. North and south type nucleosides built on a bicyclo[3.1.0]hexane template.

Among these examples, the direct correlation of conformation and its biological activity was elucidated by the elegant example of Zidovudine (AZT),[15] an anti-HIV agent that acts by executing premature chain termination of viral DNA.[16]

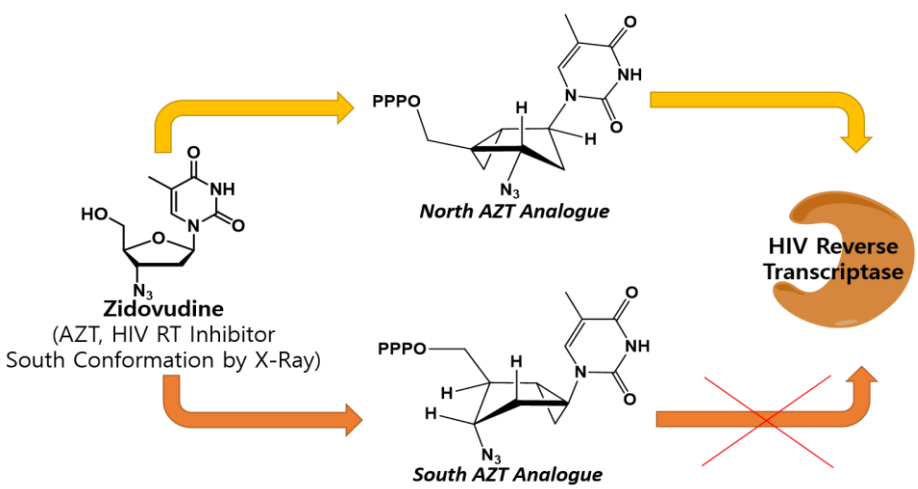


Figure 6. Graphical presentation of correlation study between conformation and anti-HIV activity of Zidovudine (AZT) analogues conducted by Marquez, V. E. and co-workers.

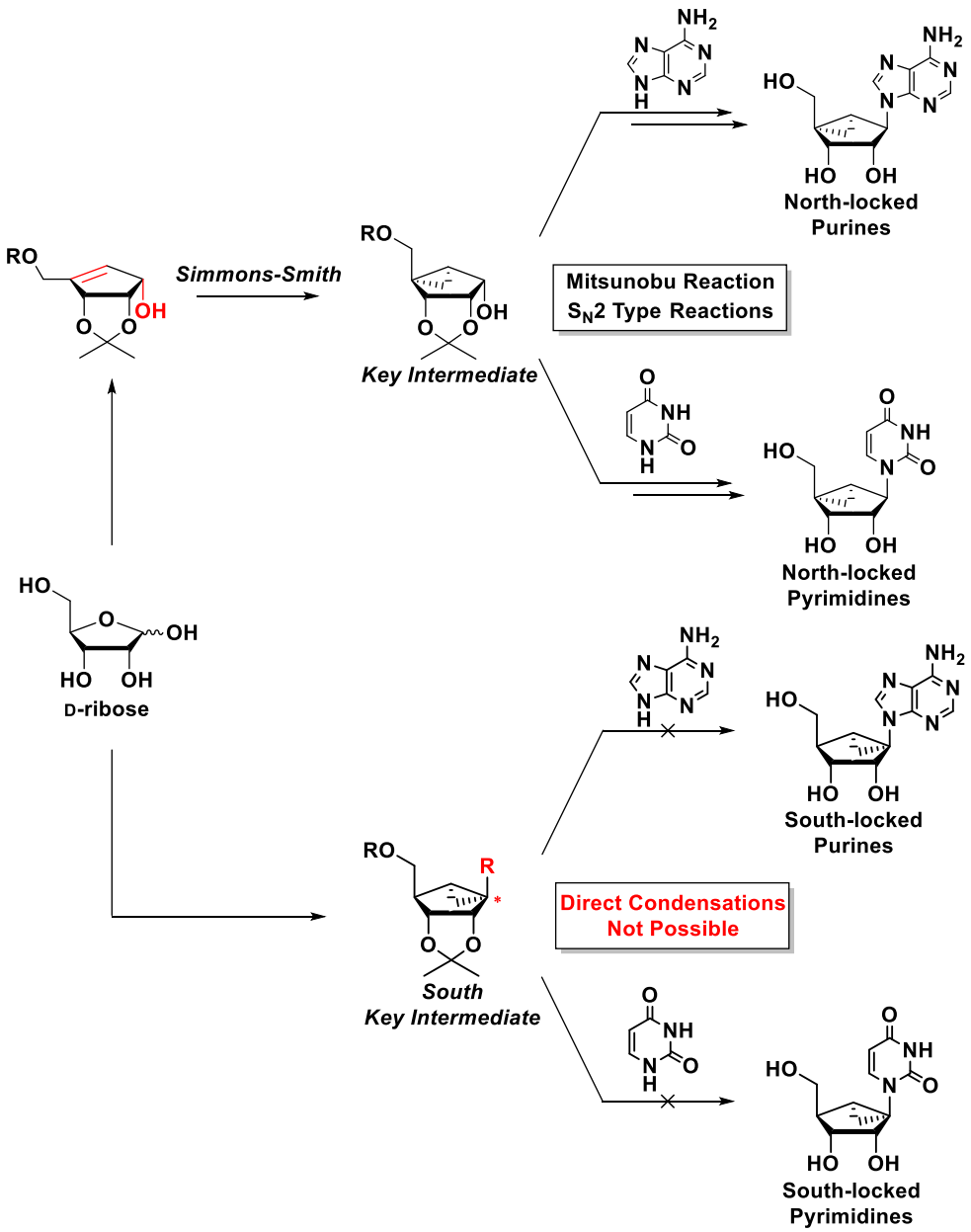
In order to test whether south conformation, as revealed by the X-ray structure of AZT, is responsible for effective antiviral activity, south and north conformation-restricted AZT analogues built on a bicyclo[3.1.0]hexane template were designed, synthesized and tested for their antiviral activity.[14] Surprisingly, only the chemically synthesized north-restricted AZT 5'-triphosphate was active against HIV whereas the expected south analogue did not inhibit HIV reverse transcriptase.[15] Further studies on herpes simplex virus showed the contrasting preferences for the two analogues,[17] indicating the each enzymes have their own conformational preferences in binding activity.

Discovery of biologically significant conformation restricted nucleosides continued.[18] A north methanocarba analogue, MRS5980, showed potent A_3AR agonist affinity compared to its south anaologue,[19] while MRS2795, a south analogue, showed selective P2Y_6R affinity.[20]

1.4 Synthesis of Bicyclo[3.1.0]hexane Template

Although the role of sugar conformation in biological activity was evident from reported literature, the synthetic difficulty of the bicyclo[3.1.0]hexane template remains one of the key challenges to overcome in conducting structural diversification for further research. The synthesis of the south analogue is significantly more challenging compared to its north counterpart.

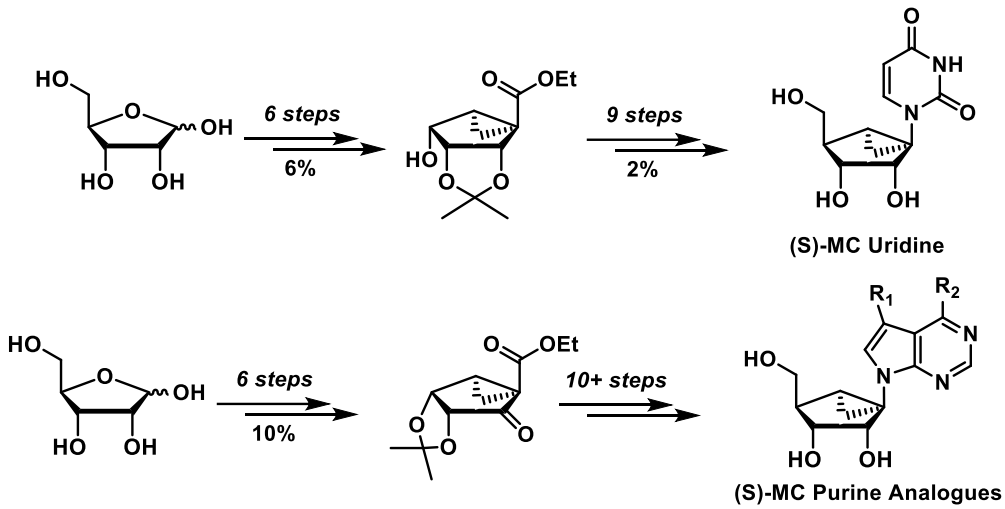
In the case of north analogue, hydroxyl-directed cyclopropanation of allylic alcohol (Figure 7.) introduces cyclopropyl moiety with the desired stereochemical outcome, at the same time conveniently installing the desired stereochemistry of the C4 substituent. This key intermediate is then capable of undergoing diverse S_N2 type reactions including Mitsunobu reactions to give variety of north methanocarba analogues.



Scheme 1. Synthetic approaches of conformation restricted nucleosides built on a bicyclo[3.1.0]hexane template.

On the other hand, the south analogue possessing a quaternary center as a α-tertiary amine prevents the nucleobase to be condensed directly. Thus, a linear base build-up approach is inevitable and an additional process for the installing the C4 stereochemistry is necessary unlike the north counterpart. Due to these reasons, only a few synthetic routes for the south

ribonucleoside analogues are reported to date.[21,22] Although the reported routes successfully gave access to south conformation-restricted pyrimidine and purine nucleosides, fact that the overall yield being less than 1%[21] still denotes a large scale preparation is still practically not feasible and thus a synthetic route with better efficiency is still in need.



Scheme 2. Known synthesis of south methanocarba uridine (top),[21] and south methanocarba purine analogues (down).[22] The overall yields of both cases were less than 1% according to the reported literatures.

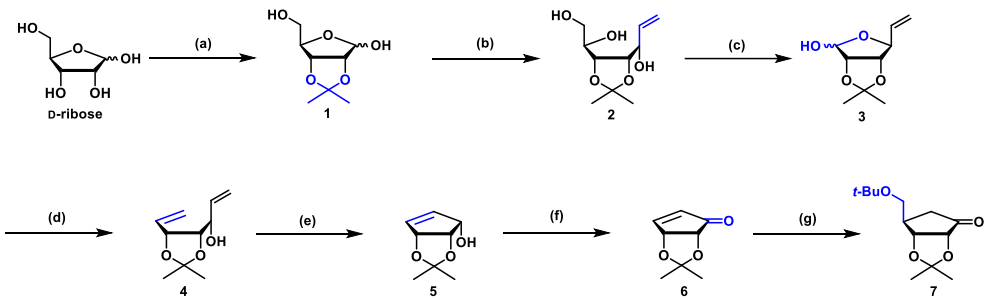
Thus, this project aims to develop an efficient route to access south methanocarba nucleosides built on a bicyclo[3.1.0]hexane template for further exploration as a therapeutic tool.

2. Synthesis of South Methanocarba Nucleosides built on a Bicyclo[3.1.0]hexane Template

2.1 Establishment of the C2, C3, and C4 Stereocenters

Since the target structure had 5 continuous stereocenters installed in a cyclopentane core, my approach towards constructing the bicyclo[3.1.0]hexane template began with preparing carbocyclic sugar moiety. The key consideration in this approach included whether the synthesis can be conducted in multigram scale, as the expected synthetic

route involved 20+ steps to the target nucleoside. Whether to start with a chiral pool or not had been one of the most important questions in the retrosynthetic analysis stage. Several possible synthetic routes starting outside of the chiral pool were proposed and a few seemed reasonable giving access, theoretically at least, to the carbocyclic core; however, the stereochemical outcomes of the proposed asymmetric reactions seemed to require an in-depth study as well as trial-and-error processes, as preliminary literature review showed inconsistent results on the hypothesis. Moreover, the reagents employed in asymmetric inductions were generally economically not feasible, thus I have decided to follow our previously published procedure in preparing the carbocyclic core[23,24] instead, with D-ribose, a naturally abundant chiral pool, as the starting material.



Scheme 3. Synthesis of the carbocyclic template. ***Reagents and conditions*:** (a) *c*-H_2SO_4, acetone, rt, 3 h; (b) vinylmagnesium bromide, THF, -78 °C to rt, 3 h; (c) $NaIO_4$, CH_2Cl_2, H_2O, 0 °C to rt; (d) CH_3PPh_3Br, THF, -78 °C to rt, 3 h; d) Neolyst M2, CH_2Cl_2, rt, 24 h; e) PDC, CH_2Cl_2, rt, 24 h, 42% for 6 steps; f) $CuLi(t\text{-}BuOCH_2)_2$, THF, -78 °C to rt, 6 h, 81%.

The conversion of readily available D-ribose to its carbocyclic template is presented in Scheme 3. Selective protection of the 2, 3-diol with acetone in the presence of catalytic sulfuric acid gave the protected D-ribose **1**, of which underwent Grignard reaction with vinylmagnesium bromide to give triol **2**. The oxidative cleavage of **2** gave lactol **3**, in turn underwent Witting reaction to give the ring-closing metathesis (RCM) precursor **4**. The RCM reaction with Neolyst M2 as the catalyst smoothly afforded the cyclopentene **5** of which underwent oxidation reaction with PDC in an one-pot manner to successfully afford cyclopentenone **6** in 42% yield from D-ribose in 6 steps.

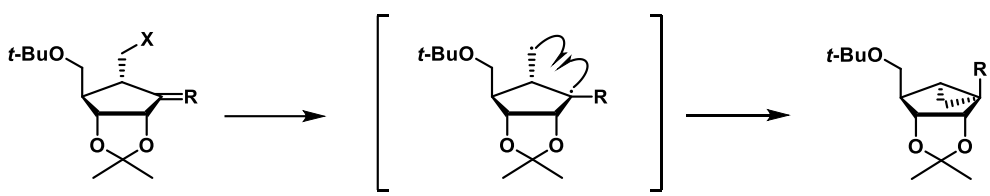
With C2 and C3 stereocenters preinstalled from the starting material, it was essential to establish the 4'-D-stereoconfiguration. This was achieved by relying on facial selectivity. 1,4-Michael addition of *tert*-butoxymethyl Gilman reagent attacking from the *β*-face induced by the sterics clash with the isopropylidene group afforded the 4'-D-configuration exclusively, resulting in the carbocyclic sugar moiety **7** in 81% yield.

2.2 Approaches in Constructing the Bicyclo[3.1.0]hexane

With the carbocyclic sugar **7** in hand, several synthetic routes to access the bicyclo[3.1.0]hexane template had been proposed. While several approaches proved to be ineffective, few synthetic routes successfully provided the bicyclo[3.1.0]hexane template.

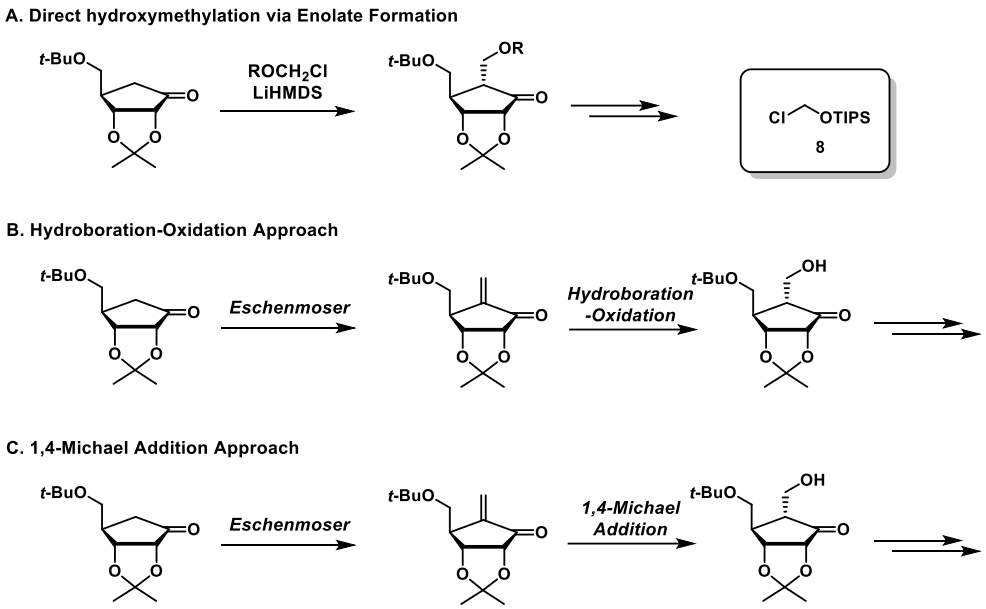
2.2.1. Radical-mediated Cyclopropanation Approach

Earlier approaches in installing the cyclopropyl group in a stereoselective manner included the use of radical as a tool to form carbon-carbon bond as depicted in Scheme 4.



Scheme 4. Description of the key reaction. A radical-mediated carbon-carbon bond formation was envisioned as the key in installing cyclopropyl group.

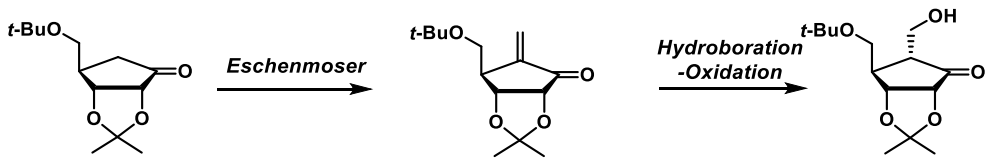
There were only a handful of literature reporting the formation of cyclopropyl unit *via* radicals, thus the proposed synthetic approach seemed worth pursuing as a novel work. In order to prepare the precursor for the key cyclopropanation reaction, a hydroxymethyl unit needed to be installed. Strategies were planned as shown in Scheme 5.



Scheme 5. Major strategies for the preparation of chiral hydroxymethyl unit.

The direct hydroxymethylation *via* enolate formation was ideal (Scheme 5. A) and was thus attempted as the priority. Due to the difficulty of preparing hydroxy-protected-methyl halide, commercially available **8** was used. The underlying expectations of this approach was 1) the enolate formation of ketone with lithium amide bases followed by the treatment of **8** would result in a α-alkylation of ketone giving rise to the desired hydroxymethylated product; and 2) due to the steric clash between the neighboring C4-*tert*-butoxymethyl group, hydroxymethyl moiety being installed would favor *trans* relationship respect to the C4 substituent, resulting in the desired *β*-stereochemistry at the key C6. Unfortunately, the α-hydroxymethylation of ketone did not proceed smoothly nor gave the desired stereochemical outcome. Many of the lithium amide bases were investigated including LiHMDS and LDA; yet, none of the results were satisfactory. Most of the cases showed formation of complex mixture and NMR analysis of the mixture of two major products seemed to be diastereomers. Unfortunately, the two were inseparable by silica gel column chromatography and thus a detailed spectral analysis of each was not carried

out. Major side product isolated was the TIPS silyl enol ether, suggesting that the silyl group should be avoided as the hydroxyl protecting group of the hydroxymethyl donor. At this point, this route has been abandoned since much efforts were required to secure the chiral template before carrying out the key cyclopropanation reaction.



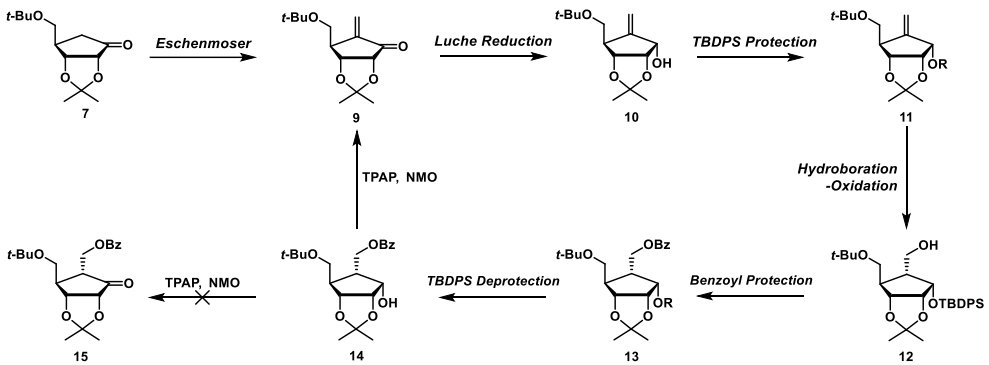
Scheme 6. Direct hydroboration-oxidation for the preparation of chiral hydroxymethyl unit.

An alternative strategy was to employ a 2-step sequence instead, *via* an *exo*-olefin intermediate, (Scheme 6) of which the *exo*-methylene moiety was easily introduced using Eschenmoser's salt. The exocyclic α,β-unsaturated ketone was found to be unstable at room temperature (although it was stable enough to be stored for few days), thus fresh batches were prepared each time before conducting further reactions.

The initial approach utilized the hydroboration-oxidation reaction (Scheme 5. B) with 9-BBN as the borating agent and sodium perborate as the reagent for oxidation, however, although the starting material was completely consumed, no major product was formed after the oxidation reaction. The same pattern was observed with different borating agents such as dicyclohexylborane, bis(pinacolato)diboron, borane-THF complex and hydrogen peroxide, aqueous sodium hydroxide as oxidizing agents. A possible explanation for such phenomenon was based on the hypothesis that the ketone was also being influenced by the borating process resulting in the formation of multiple products.

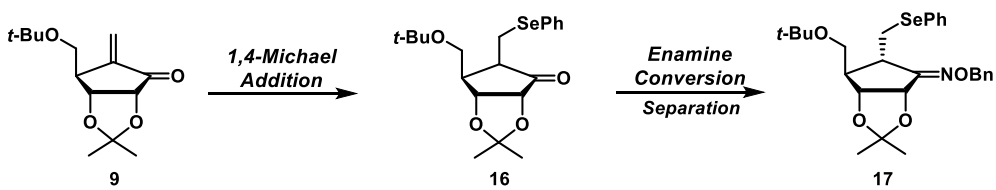
In order to test the hypothesis, a stereoselective Luche reduction was conducted and the resulting secondary hydroxyl group was protected with TBDPS (Scheme 7). The hydroboration-oxidation reaction of **11** produced

the product with the desired stereochemistry along with minor formation of its diastereomer, suggesting that the ketone was affecting earlier attempts. Protection of the hydroxyl group with benzoyl furnished **13**, which underwent silyl deprotection with TBAF to furnish **14**. At this point, still considerable number of steps, including oxidation, enamine conversion, deprotection of the benzoyl group and halogenation of the resulting alcohol, were required to access the cyclopropanation precursor.



Scheme 7. Revised hydroboration-oxidation route.

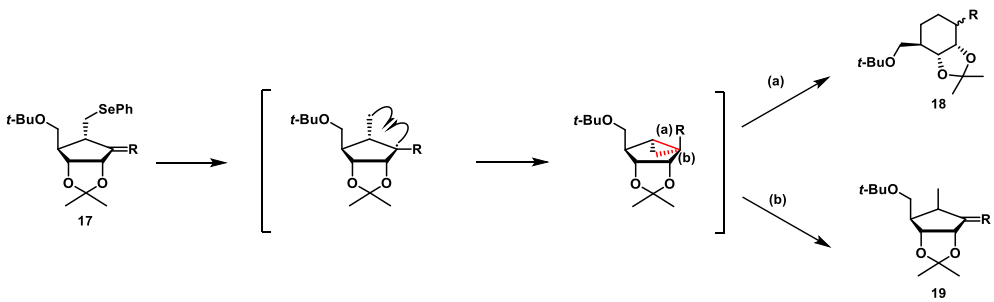
Furthermore, the TPAP oxidation of **14** surprisingly resulted in its earlier intermediate **9** instead of the desired product **15** prompted me to seek for an alternative route as the approaches thus far could barely provide the product for the key cyclopropanation reaction.



Scheme 8. 1,4-Michael addition route for radical-mediated cyclopropanation precursor.

In order to test whether the time and effort in preparing the cyclopropanation precursor is worth investing, a third approach has been applied (Scheme 8) to quickly try out the key reaction. The 1,4-Michael

addition was expected to give poor stereoselectivity; however, it was the easiest route to access the cyclopropanation precursor. After *exo*-methylenation, 1,4-Michael addition with diphenyldiselenium successfully afforded the addition product **16** in an excellent yield of 92%. The mixture was then treated the benzylamine hydrochloride to give enamine **17**. At this stage the diastereomers were separable. With the key precursor **17** in hand, the radical-mediated cyclopropanation was carried out.



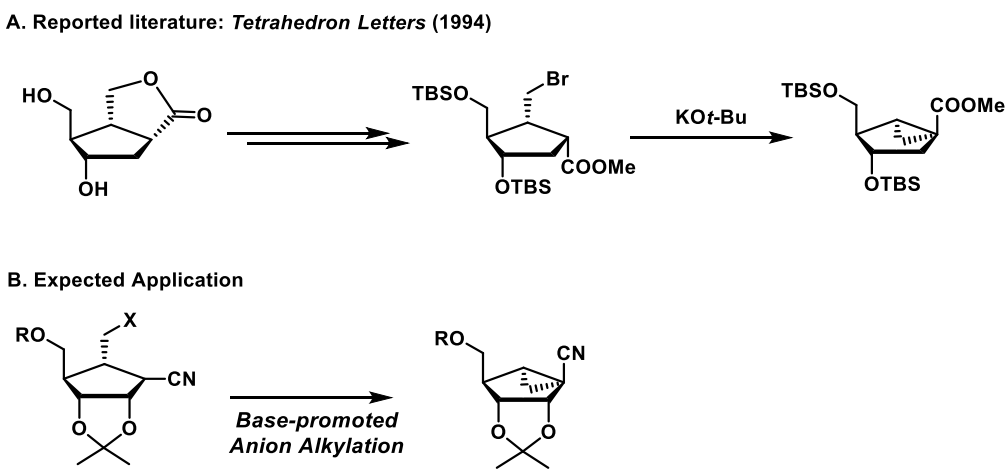
Scheme 9. Results of the key radical-mediated cyclopropanation reaction

Two major products formed, of which neither was the desired product. NMR analysis of the two major products revealed that one was the cyclohexyl sugar **18** and the other to be *exo*-methyl product **19**. Plausible mechanistic explanation involves the formation of the bicyclo[3.1.0]hexane template followed by additional undesired radical expansion reaction as shown in Scheme 9. Samarium diiodide, tributyltin hydride and triethyl borane as the radical source all failed to provide the desired bicyclic product. Reaction at lower temperature also proved to be ineffective. Radical-mediated cyclopropanation approach was not investigated any further.

2.2.2. Cyclopropanation by Anion Alkylation Approach

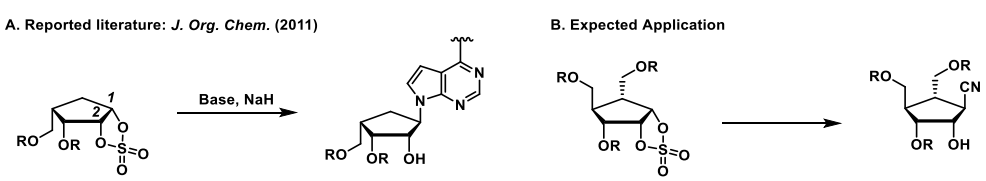
As radical-mediated approach was unsuccessful, and with the hydroxymethyl intermediate in hand, an attempt to conduct cyclopropanation *via* anion alkylation was investigated based on a reported case of deoxy south nucleoside synthesis introducing the cyclopropyl group *via* anion alkylation (Scheme 10).[25] Thus, creation of an acidic proton at C1

was the key. Nitrile group was chosen to achieve such goal, thus ketone needed to be converted into nitrile without the formation of enolate as it will destroy the C6 stereocenter. Van Leusen reaction[26] seemed promising, yet in the given system the desired product was not observed. Cyanohydrin formation was found to be a reversible process, reverting to its ketone, resulting in a poor isolated yield.



Scheme 10. Reported protocol for the synthesis of deoxy south nucleoside *via* anion alkylation as the key step (top).[25] Based on the reported literature, synthetic route has been designed.

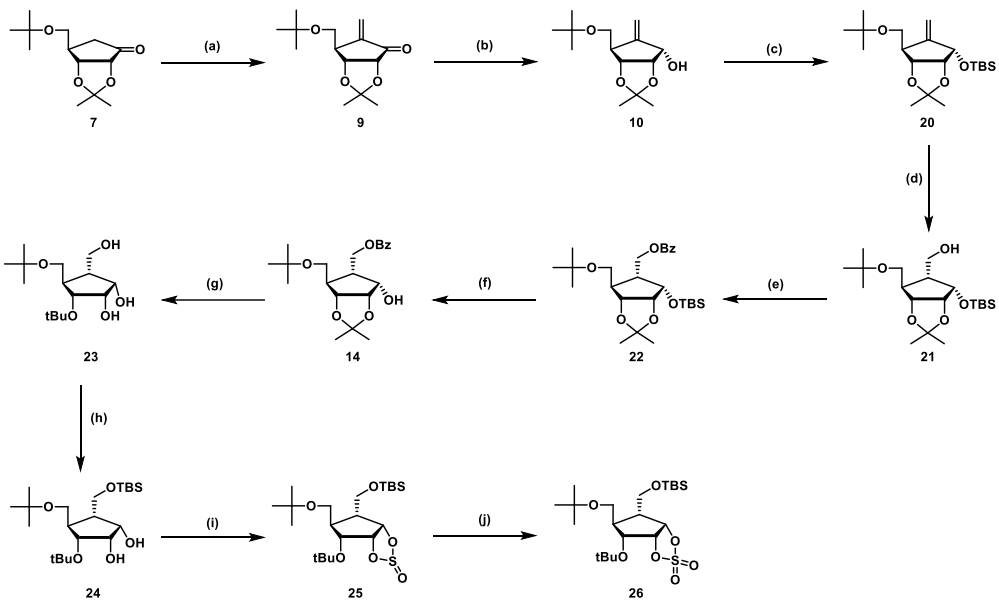
An alternative approach in installing the nitrile was to utilize the cyclic sulfate as an epoxide surrogate as shown in Scheme 11. Lee, H. W., *et. al.* has reported the condensation of a nucleobase using regioselective cyclic sulfate ring opening reaction, [27] which I thought would be useful in introducing the nitrile group.



Scheme 11. Regioselective cyclic sulfate chemistry

The hydroxymethyl-installed intermediate **14** was prepared analogous to

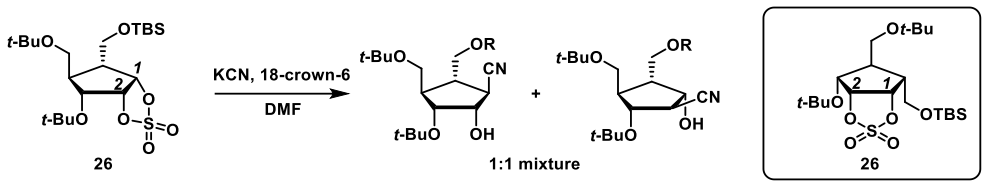
the previous approaches. Trimethylaluminum-mediated regioselective isopropylidene cleavage unintendedly deprotected the benzoyl group to afford triol **23**, of which the primary hydroxyl group was selectively re-protected with TBS group to give diol **24**. The cyclic sulfate moiety was prepared by reacting the diol with thionyl chloride, followed by the oxidation of cyclic sulfite **25** to its cyclic sulfate **26** using sodium periodate in the presence of catalytic amount of ruthenium chloride.



Scheme 11. Preparation of the anion-alkylation precursor. Reagents and conditions: (a) Eschenmoser's salt, LiHMDS, THF, -78 °C, 2 h, 76%; (b) $CeCl_3 \cdot 7H_2O$, $NaBH_4$, MeOH, 0 °C, 30 min, 86%; (c) TBSCl, imidazole, CH_2Cl_2, rt, 16 h, 81%; (d) i) BH_3-THF complex, ii) $NaBO_3 \cdot H2O$, 0 °C to rt, 16 h, 81%; (e) BzCl, DMAP, Et_3N, CH_2Cl_2, 0 °C to rt, 16 h, 91%; (f) TBAF, AcOH, THF, 0 °C to rt, 20 h, 83%; (g) $AlMe_3$, CH_2Cl_2, 0 °C to rt, 36 h; 50%; (h) $SOCl_2$, Et_3N, CH_2Cl_2, 0 °C, 1 h; (i) $RuCl_3 \cdot xH_2O$, $NaIO_4$, CCl_4/MeCN/H_2O, rt.

With cyclic sulfate **26** in hand, ring opening of the cyclic sulfate was achieved by employing potassium cyanide in the presence of 18-crown-6. Unfortunately, the displacement of the nitrile showed no regioselectivity, resulting in a 1:1 mixture. Unlike the reported literature, the system in this case had nearly a symmetrical structure in respect to the displacement site, showing no distinguishable factors for the nucleophilic attack (the rotated

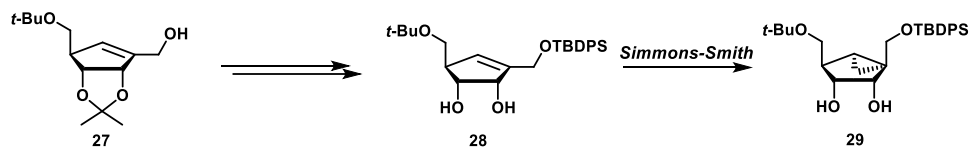
view of 26 shown in Scheme 12 shows nearly symmetrical structure respect to the displacement sites C1 and C2). This result indicates the regioselectivity of the cyclic sulfate ring opening is greatly influenced by neighboring substituent effects, rather than steric factors.



Scheme 12. Experimental results of nitrile displacement to cyclic sulfate. Rotated view (right box) shows that the displacement sites are nearly symmetrical.

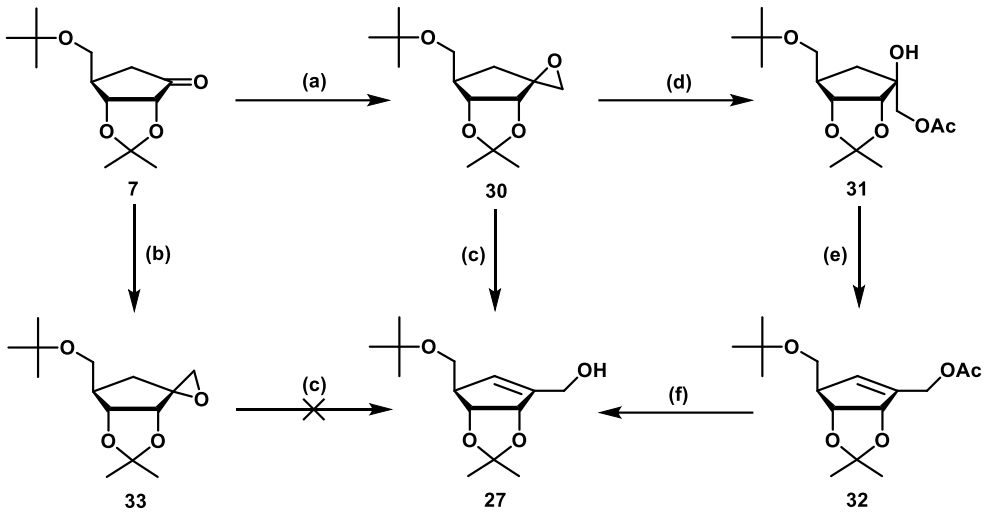
2.2.3. Hydroxy-directed Simmons-Smith Cyclopropanation Approach

The focus shifted to classic Simmons-Smith cyclopropanation reaction. The key idea of this route was based on earlier literatures reporting the directing effect of the hydroxyl group in Simmons-Smith cyclopropanation reaction.[28] Allylic alcohol **27** was designed to be the target compound as the precursor for the cyclopropanation reaction (Scheme 13).



Scheme 13. Key reaction for hydroxy-directed Simmons-Smith Cyclopropanation approach.

The preparation of allylic alcohol started with the carbocyclic template **7**. Corey-Chaykovsky epoxidation successfully provided *spiro*-epoxide **30** in excellent yield; however, Lewis acid-mediated rearrangement of the *spiro*-epoxide to its allylic alcohol was sluggish. Aluminum isopropoxide in refluxing toluene for 72 hours furnished the desired allylic alcohol in a yield of 63% based on the recovery of starting material.



Scheme 14. Preparation of allylic alcohol **27**. ***Reagents and conditions***: (a) $Me_3S(O)I$, NaH, DMSO, 0 ºC to rt, 10 min, 81%; (b) CH_2Br_2, *n*-BuLi, THF, -78 ºC, 1 h, 70%; (c) $Al(O\text{-}iPr)_3$, PhMe, 110 ºC, 8 d, 63% (brsm); (d) KOAc, AcOH, 120 ºC, 2 h, 84%; (e) $POCl_3$, DMAP, CH_2Cl_2, 0 ºC, 30 min, 80%; (f) K_2CO_3, MeOH, rt, 1 h, 92%.

Clean conversion was not observed with prolonged reaction time. Different aluminum reagents, TBSOTf/DBU or 2,6-lutidine conditions also failed to give clean conversion. In solving such issue, conformational analysis was conducted, and a hypothesis was proposed claiming that the stereochemistry of the epoxide could have played a role in determining the rate of the rearrangement process (Figure 7). Thus, *spiro*-epoxide with opposite stereochemistry **33** was synthesized and its rearrangement to allylic alcohol has been explored; however, disappointing results were obtained. Interestingly, reacting **33** with aluminum isopropoxide did not furnish the allylic alcohol **27**. Different conditions including LDA, LiHMDS/HMPA, DATMP, and DBU all failed to give **27**. When **33** was exposed to potassium acetate in acetic acid, an identical condition of converting **30** to **31**, a significant side product formed. The α-spiro epoxide was inferior to the corresponding *β-spiro*-epoxide in every way in accessing the allylic alcohol **27**. Employing cesium acetate instead of potassium acetate for the ring opening reaction of **33** proceeded without any formation of side products

however.

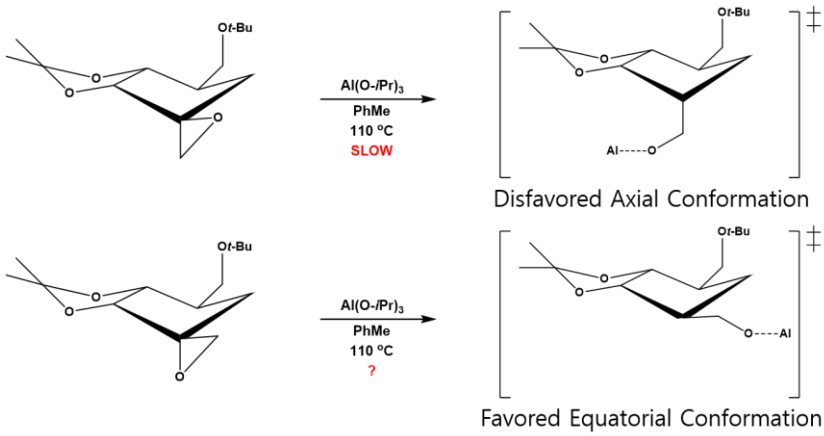
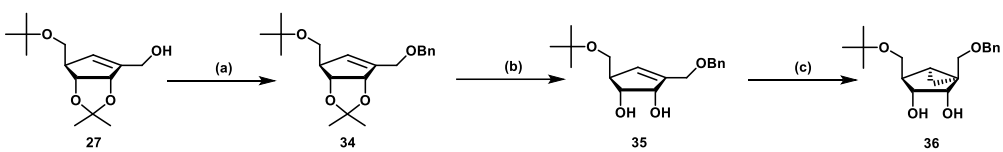


Figure 7. Hypothesis on the relationship between the stereochemistry of spiro epoxide and its influence on rearrangement to allylic alcohol.

Sufficient amount of allylic alcohol for further steps was secured by the routes, one from aluminum isopropoxide rearrangement (re-running the reaction after starting material recovery) and a 4-step sequence reported by Jung *et. al.* in his work on the total synthesis of Neplanocin A.[29] While the latter was few steps more, the reactions were high yielding with shorter reaction time.



Scheme 15. Hydroxy-directing Simmons-Smith cyclopropanation reaction. ***Reagents and conditions***: (a) BnBr, NaH, TBAI, DMF, 0 °C to rt, 4 h, 95%; (b) $CeCl_3 \cdot 7H_2O$, NaI, CH_3CN, 70 °C, 16 h, 72%; (c) Et_2Zn, CH_2I_2, CH_2Cl_2, 0 °C to rt, 7 d, 61% (brsm).

With allylic alcohol **27** in hand, Simmons-Smith cyclopropanation reaction was attempted without deprotecting the 2,3-isopropylidene, and as expected the reaction showed poor selectivity of 2:1 suggesting that the directing effect is necessary for asymmetric induction. Thus, the allylic alcohol was protected with benzyl (Bn) and the isopropylidene group was removed. The removal of isopropylidene group was achieved in

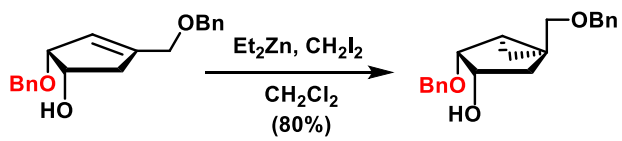
$CeCl_3 \cdot 7H_2O$/NaI system as acid, PPTS, catalyzed deprotection only yielded the desired product X in a low yield of 34%.

Surprisingly the Simmons-Smith cyclopropanation barely produced the desired product **36**, in 16% isolated yield (61% based on recovery of the starting material) after 7 days of reaction time. Many different conditions with different cyclopropanating reagents (Table 1) were attempted only to result in partial conversion or decomposition. Even an attempt with highly reactive carbenoid zinc reagent, the Shi zinc reagent,[30] was unsuccessful.

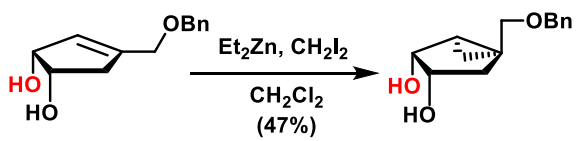
Reagents	Solvent	Temp.	Result
Et_2Zn, CH_2I_2	CH_2Cl_2	0° to rt	Partial Product
Et_2Zn, CH_2I_2	Toluene	0° to rt	Partial Product
Et_2Zn, CH_2I_2	1,2-DCE	Heating up to 40 °C	Decompose
$CF_3CO_2ZnCH_2I$, CH_2I_2	CH_2Cl_2	0° to rt	Decompose
Sm / $HgCl_2$, $ClCH_2I$	THF	0° to rt	Partial Product

Table 1. Reaction conditions attempted for cyclopropanation reaction.

While seeking for a solution to the challenge, two literature came across while doing literature reviews. These had identical system, one with an allylic and homoallylic alcohols in the same system[31] whereas the other had the allylic alcohol protected.[32] (Figure 8)



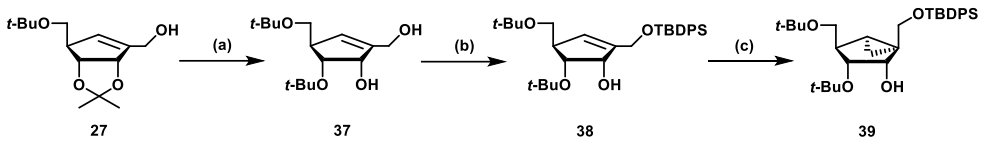
Marquez, V. E., *et. al. Helvecta Chimica Acta.* **1999**, *82*, 2119.



Comin, M. J. *et. al. J. Am. Chem. Soc.* **2007**, *129*, 6216

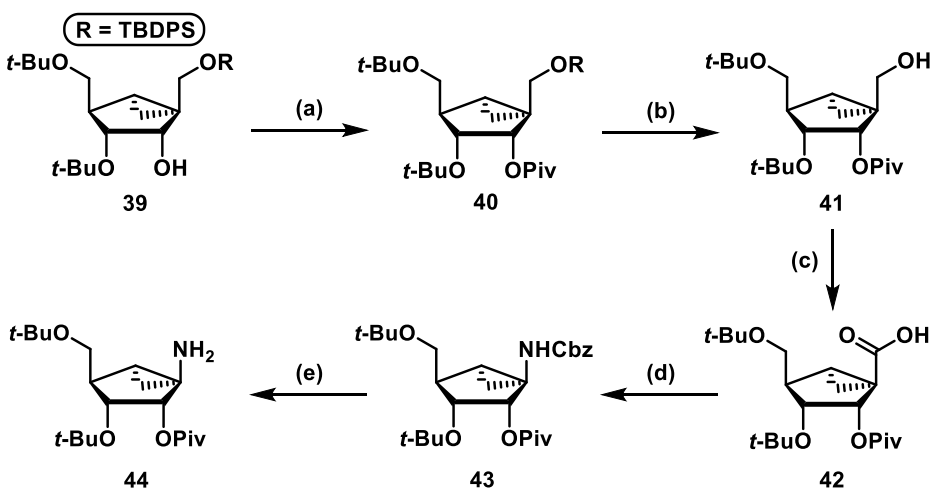
Figure 8. Results from two different literature showing the presence of free allylic and homoallylic alcohols in a same system causing significant impact on Simmons-Smith cyclopropanation reaction.

According to these reports, the presence of allylic and homoallylic alcohols in the same system showed significantly different behaviour in the Simmons-Smith cyclopropanation reaction. This is presumably due to both alcohols being chelated with zinc reagent, hindering each other from installing the cyclopropyl unit.



Scheme 16. Revised route for Simmons-Smith cyclopropanation. ***Reagents and conditions***: (a) Me_3Al, -78 ºC to rt, 5 d, 57%; (b) TBDPSCl, DMAP, Et_3N, CH_2Cl_2, 0 ºC, 3 h, 72%; (c) Et_2Zn, CH_2I_2, CH_2Cl_2, 0 ºC to rt, 5 h, 81%.

Thus, it was necessary to selectively protect one hydroxyl group in the presence of another. In order to do so, trimethylaluminum-mediated regioselective isopropylidene cleavage has been employed. The regioselective cleavage resulted in the 2'-free hydroxy substrate **37**, of which underwent Simmons-Smith cyclopropanation reaction to successfully furnish the bicyclo[3.1.0]hexane template **39** in good yields, indirectly proving that the free diol was causing the problem in cyclopropanation.

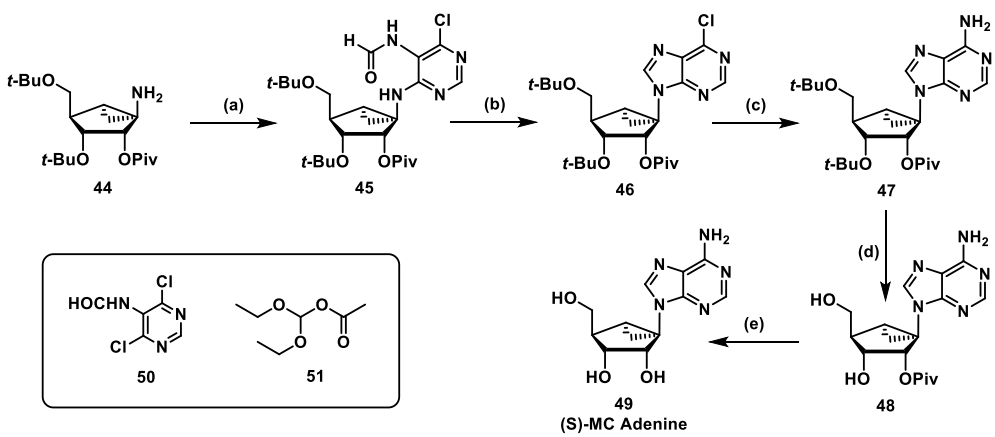


Scheme 17. Access to key intermediate **44**. ***Reagents and conditions***: (a) PivCl, DMAP, Et_3N, CH_2Cl_2, rt, 48 h, 74%; (b) TBAF, AcOH, THF, rt, 30 h, 80%; (c) $RuCl_3 \cdot xH_2O$, $NaIO_4$, CCl_4/MeCN/H_2O, rt, 70%; (d) i) DPPA, Et3N, 0 ºC to rt, ii) BnOH, PhMe, 110 ºC, 3 h, 78%; (e) H_2, Pd/C, MeOH, rt, 16 h, 99%.

The synthesis towards the key intermediate is shown in Scheme 17. Protection of the secondary hydroxyl group with pivaloyl followed by deprotection of the TBDPS furnished **41**. Ruthenium-catalyzed oxidation of **41** surprisingly resulted in concomitant oxidation of 4'-*tert*-butylmethyl ether to its ester, while PDC and other oxidations to acid proved to be ineffective. Thus, the reaction was carried out in a lower temperature with exactly 2 equivalents of sodium periodate to afford the desired acid **42**. Curtius rearrangement followed by capturing the isocyanate with benzyl alcohol furnished the benzyl carbamate **43** in excellent yield. Hydrogenation of **43** cleanly provided the key intermediate **44** which was pure enough to be used in further reactions with a simple filtration work up. Despite long steps, current route secured the bicyclo[3.1.0]hexane key intermediate **44**.

2.3. Synthesis of South Conformation-locked Nucleosides built on a Bicyclo[3.1.0]hexane

2.3.1. Synthesis of South Methanocarba Adenine

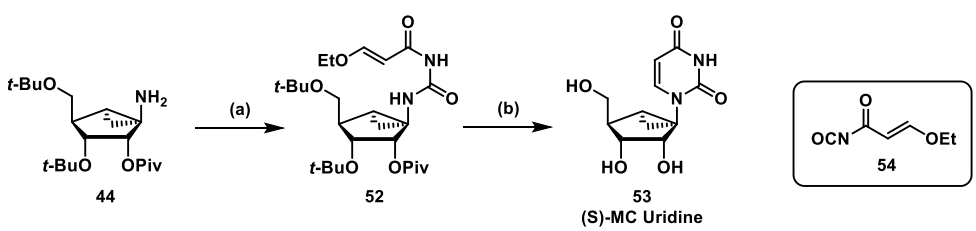


Scheme 18. Synthesis of south methanocarba adenine. ***Reagents and conditions***: (a) **50**, DIPEA, 1,4-dioxane, 100 ºC (MW), 8 h, 76%; (b) **51**, neat, 120 ºC (MW), 5 h, 90%; (c) NH_4OH, 1,4-dioxane, 100 ºC (MW), 1 h, 70%; (d) TFA:H_2O (2:1), rt to 40 ºC, 48 h, 61%; (e) NaOMe, MeOH, 0 ºC to rt, 1 h, 72%.

With the key intermediate **44** in hand, the synthesis of south

methanocarba adenine was achieved following a reported procedure.[33] The key intermediate **44** was reacted with **50** in the presence of DIPEA in 1,4-dioxane at 100 ºC under microwave irradiation to provide intermediate **45** in 76% yield. Completion of the 6-chloropurine moiety was achieved by cyclizing the foramide moiety with diethoxymethyl acetate to give the protectect 6-chlorpurine nucleoside **46**. Amination was achieved with ammonium hydroxide, where the formation of hypoxanthine was not observed. Unfortunately, the pivaloyl group was not removed during this reaction. Acidic hydrolysis of tert-butyl groups was achieved by TFA, followed by deprotection of the pivaloyl finally gave access to the target south methanocarba adenine **49**.

2.3.2. Synthesis of South Methanocarba Uridine



Scheme 19. Synthesis of south methanocarba uridine. ***Reagents and conditions***: (a) **54**, CH_2Cl_2, 0 ºC to rt, 68%; (b) HCl, THF, 80 ºC, 4 h, 61%.

Synthesis of south methanocarba uridine followed a general procedure from its north counterpart.[34] Key intermediate **44** was reacted with isocyanate **54**, fresh prepared by the known protocol[35] furnished intermediate **52**. The ethanol HCl condition resulted in a trace amount of **53** along with the formation of complex mixture. However, the reaction went smoothly when ethanol was replaced with THF. Acid-catalyzed cyclization and global deprotection in one-pot furnished the target south methanocarba uridine **53**.

3. Conclusion

Synthesis of south conformation-locked adenine and uridine built on a bicyclo[3.1.0]hexane template was achieved. Diverse routes were explored, and experimental results that may find its usefulness in future application has been obtained.

4. Experimental Section

General Methods

Proton (^{1}H), carbon (^{13}C) NMR, spectra were recorded on a Bruker AV 400 (400/100 MHz), Bruker AMX 500 (500/125 MHz), or Jeol JNM-ECA600 (600/150 MHz). Chemical shifts are given in parts per million (ppm) (δ) relative to the solvent peak. Coupling constants (J) are reported as Hertz (Hz). High resolution mass spectra (HRMS) measurements were performed on a Thermo LCQ XP instrument. Optical rotations were determined on Jasco III in appropriate solvent. UV spectra were obtained on U-3000 made by Hitachi in methanol or water. Melting points were determined on a Buchan B-540 instrument and are uncorrected. Reactions were checked by thin layer chromatography (Kieselgel 60 F254, Merck). Spots were visualized under UV light (254 nm), or by colorizing and charring with a *p*-anisaldehyde solution or a phosphomolybdic acid solution. The crude compounds were purified by silica gel column chromatography (Kieselgel 60, 70-230 mesh, Merck). All the anhydrous solvents were redistilled over CaH_2, P_2O_5, or sodium/benzophenone before the reaction. All reactions were performed under nitrogen atmosphere and anhydrous solvents unless specified otherwise.

(1*R*,2*S*,3*R*,4*R*,5*S*)-3-(*tert*-butoxy)-4-(*tert*-butoxymethyl)-1-(((*tert*-butyldi-phenylsilyl)oxy)methyl)bicyclo[3.1.0]hexan-2-yl pivalate (40)

To a stirred solution of **39** (92 mg, 0.18 mmol), DMAP (2.4 mg, 0.02 mmol) and triethylamine (0.05 mL, 0.36 mmol) in dry dichloromethane (5 mL) was added pivaloyl chloride (0.05 mL, 0.36 mmol) dropwise at 0 ºC and the resulting reaction mixture was stirred at room temperature for 48 hours under nitrogen atmosphere. After completion of reaction observed by TLC, the volatiles were removed *in vacuo* and the residue was purified by silica gel column chromatography to afford title compound **40** (81 mg, 74%) as a colorless oil.

(1*R*,2*S*,3*R*,4*R*,5*S*)-3-(*tert*-butoxy)-4-(*tert*-butoxymethyl)-1-(hydroxymethyl)bicyclo[3.1.-0]hexan-2-yl pivalate (41)

To a stirred solution of **40** (81 mg, 0.13 mmol) in dry tetrahydrofuran (5 mL) was dropwise added a premade mixture of TBAF (0.2 mL, 0.20 mmol, 1.0M solution in THF) and acetic acid (20:1 v/v) at 0 ºC under nitrogen atmosphere and the resulting reaction mixture was stirred at room temperature for 24 hours. The reaction was quenched with saturated aqueous ammonium chloride solution and poured into water (10 mL). The aqueous layer was extracted with ethyl acetate (10 mL x 2) and the combined organic layer was washed with brine, dried over anhydrous magnesium sulfate and concentrated in vacuo. The crude reisude was purified by silica gel column chromatography to afford title compound **41** (39 mg, 80%) as a colorless oil.

(1*S*,2*S*,3*R*,4*R*,5*S*)-3-(*tert*-butoxy)-4-(*tert*-butoxymethyl)-2-(pivaloyloxy)-bicyclo[3.1.0]-]h-exane-1-carboxylic acid (42)

To a stirred solution of **41** (90 mg, 0.24 mmol) in MeCN/CCl_4/H_2O (4.5 mL, 2:2:3 v/v) was added ruthenium chloride hydrate (4 mg ,0.02 mmol), followed by sodium periodiate (103 mg, 0.48 mmol) and the resulting

reaction mixture was stirred at 0 ºC for 4 hours. The reaction was filtered through a pad of Celite, and the resulting mixture was concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography eluting hexane/ethyl acetate (1:1) with 1 mL of acetic acid in every 100 mL of eluting solvents to afford the title compound **42** (65 mg, 70%) as a colorless oil.

(1*S*,2*S*,3*R*,4*R*,5*S*)-1-(((benzyloxy)carbonyl)amino)-3-(*tert*-butoxy)-4-(*tert*-butoxymethyl)-bicyclo[3.1.0]hexan-2-yl pivalate (43)

To a stirred solution of **42** (65 mg, 0.17 mmol) in dry toluene (5 mL) was added triethylamine (0.03 mL, 0.20 mmol) followed by DPPA (0.04 mL, 0.20 mmol) at 0 ºC and the resulting solution was stirred under nitrogen atmosphere for 30 minutes. To the solution was then added benzyl alcohol (0.03 mL, 0.26 mmol) and the resulting reaction mixture was heated to reflux and stirred for 4 hours. The reaction was cooled room temperature and the volatiles were removed in vacuo. The crude residue was purified by silica gel column chromatography to afford the title compound **43** (65 mg, 78%) as a colorless oil.

(1*S*,2*S*,3*R*,4*R*,5*S*)-1-amino-3-(*tert*-butoxy)-4-(*tert*-butoxymethyl)bicyclo[3.1.0]hexan-2-yl pivalate (44)

To a stirred solution of **43** (65 mg, 0.13 mmol) in methanol (10 mL) was added palladium on activated carbon (1 mg, 0.01 mmol) and the resulting reaction mixture was stirred under an atmosphere of hydrogen for 16 hours. The crude residue was filtered through a pad of Celite, and the volatiles were removed in vacuo. The crude residue **44** (46 mg, 99%) was used directly without any further purification.

(1*S*,2*S*,3*R*,4*R*,5*S*)-3-(*tert*-butoxy)-4-(*tert*-butoxymethyl)-1-((6-chloro-5-formamidopyrim-idin-4-yl)amino)bicyclo[3.1.0]hexan-2-yl pivalate (45)

To a stirred solution of **44** (46 mg, 0.13 mmol) in 1,4-dioxane (3 mL) was added DIPEA (0.10 mL,0.52 mmol) followed by *N*-(4,6-dichloropyrimidin-5-yl)formimidic acid (27 mg, 0.14 mmol) and the reaction was heated and stirred at 100 ºC for 8 hours under microwave irradiation. The resulting mixture was concentrated *in vacuo*, and the crude residue was purified by silica gel column chromatography to afford the title compound **45** (51 mg, 76%) as a colorless oil.

(1*S*,2*S*,3*R*,4*R*,5*S*)-3-(*tert*-butoxy)-4-(*tert*-butoxymethyl)-1-(6-chloro-9*H*-purin-9-yl)bicy-clo[3.1.0]hexan-2-yl pivalate (46)

Compound **45** (51 mg, 0.10 mmol) was dissolved in diethoxymethyl acetate (3 mL) and the reaction was stirred under microwave irradiation at 120 ºC for 5 hours. After cooling to room temperature, the volatiles were removed in vacuo and the crude residue was purified by silica gel column chromatography to afford the title compound **46** (43 mg, 90%) as a white solid.

(1*S*,2*S*,3*R*,4*R*,5*S*)-1-(6-amino-9*H*-purin-9-yl)-3-hydroxy-4-(hydroxymet-hyl)bicyclo[-3.1.0]hexan-2-yl pivalate (48)

Compound **46** (43 mg, 0.09 mmol) was dissolved in TFA/H_2O (3 mL, 2:1 *v/v*) and the resulting reaction mixture was stirred at 40 ºC for 48 hours. The volates were removed *in vacuo* and the crude residue was purified by silica gel column chromatography to afford the title compound **48** (19.8 mg ,0.05 mmol) as a white solid.

(1*S*,2*S*,3*R*,4*R*,5*S*)-1-(6-amino-9*H*-purin-9-yl)-4-(hydroxymethyl)bicyclo-[3.1.0]hexane-2,3-diol (49)

To a stirred solution of **48** (19.8 mg, 0.05 mmol) in methanol (3 mL) was added sodium methoxide (5.4 mg, 0.10 mmol) at 0 ºC and the resulting reaction mixture was stirred at 0 ºC under nitrogen atmosphere for 1 hour. The volatiles were then removed in vacuo and the crude residue was purified by silica gel column chromatography to afford the title coumpound **49** (10 mg, 72%) as a white solid.

(1*S*,2*S*,3*R*,4*R*,5*S*)-3-(*tert*-butoxy)-4-(*tert*-butoxymethyl)-1-(3-((*E*)-3-etho-xyacryloyl)urei-do)bicyclo[3.1.0]hexan-2-yl pivalate (52)

To a stirred solution of **44** (24 mg, 0.07 mmol) in dry dichloromethane (3 mL) was dropwise added freshly prepared (*E*)-3-ethoxyacryloyl isocyanate 0.4M solution in toluene (0.35 mL, 0.14 mmol) at 0 ºC under nitrogen atmosphere and the resulting reaction mixture was stirred at room temperature for 4 hours. The volatiles were removed in vacuo and the crude residue was purified by silica gel column chromatography to afford the title compound **52** (24 mg, 68%) as a colorless oil.

1-((1*S*,2*S*,3*R*,4*R*,5*S*)-2,3-dihydroxy-4-(hydroxymethyl)bicyclo[3.1.0]hex-an-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (53)

Compound **52** (24 mg, 0.05 mmol) was dissolved in a small amount of tetrahydrofuran (0.5 mL) and 2*N* HCl solution (3 mL) was added. The resulting solution was then heated to 80 ºC and stirred for 4 hours. The reaction was cooled to room temperature and the volatiles were removed *in vacuo*. The crude residue was purified by silica gel column chromatography to afford the title compound **53** (8 mg, 61%) as a white solid.

5. References

1. Watson, J. D., Crick, F. H. *Nature*, **1953**, *171*, 737.
2. Rutherford, A. DNA double helix: discovery that led to 60 years of biological revolution. **2013**. *The Guardian*. https://www.theguardian.com/science/2013/apr/25/dna-double-helix-60-years-biological-revolution (Accessed June 27, 2019).
3. Agrawal, S., Gait, M. J. History and Development of Nucleotide Analogues in Nucleic Acids Drugs. In *Advances in Nucleic Acid Therapeutics.*; Thurston, D., Ed.; Drug Discovery Series 68; Royal Society of Chemistry: Croydon, UK, 2019; pp 1-21.
4. (a) Saenger, W. *Principles of Nucleic Acid Structure*, Springer-Verlag, **1984**.; (b) Rich, A. *Nat. Struct. Bio*. **2003**, *10*, 247.; Patel, D. J., Shen, C. *Proc. Natl. Acad. Sci*. **1978**, *6*, 2553.; (c) Williams, A. A., Darwanto, A., Theruvathu, J. A., Burdzy, A., Neidigh, J. W., Sowers, L. C. *Biochemistry* **2009**, *48*, 11994.
5. Anosova, I., Kowal, E. A., Dunn, M. R., Chaput, J. C., Van Horn, W. D., Egli, M. *Nucleic Acids Research* **2016**, *44*, 1007.
6. Kilpatrick, J., Pitzer, K. Spitzer, R. *J. Am. Chem. Soc.* **1947**, *69*, 2483.
7. Ptizer, K., Donath, W. E. *J. Am. Chem. Soc.* **1959**, *81*, 3213.
8. Altona, C., Sundaralingam, M. *J. Am. Chem. Soc.* **1972**, *94*, 8205.
9. Altona, C., Geise, H. J., Romers, C. *Tetrahedron*, **1968**, *24*, 4377.
10. DeLeeuw, H. P. M., Haasnoot, C. A. G., Altona, C. *Isr. J. Chem.* **1980**, *20*, 108.
11. Marquez, V. E., Ezzitouni, A., Russ, P., Siddiqui, M. A., Ford, Jr., H., Feldman, R. J., Mitsuya, H., George, C., Barchi, Jr., J. J. *J. Am. Chem. Soc.* **1998**, *120*, 2780.
12. Kinoshita, K., Yaginuma, S., Hayashi, M., Nakatsu, K. *Nucleosides & Nucleotides*, **1985**, *4*, 661.
13. Rodriguez, J. B., Marquez, V. E., Nicklaus, M. C., Barchi, Jr., J. J. *Tetrahedron Lett.*, **1993**, *34*, 6233.

14. (a) Rodriguez, J. B., Marquez, V. E., Nicklaus, M. C., Mitsuya, H., Barchi, Jr., J. J. *J. Med. Chem.*, **1994**, *37*, 3389.; (b) Marqeuz, V. E., Siddiqui, M. A., Ezzitouni, A., Russ, P., Wang, J., Wagner, R. W., Matteucci, M D. *J. Med. Chem*. **1996**, *39*, 3739.
15. Marquez, V. E., Ezzitouni, A., Russ, P., Siddiqui, M. A., Ford, Jr., H., Feldman, R. J., Mitsuya, H., George, C., Barchi, Jr., J. J. *J. Am. Chem. Soc*. **1998**, *120*, 2780.
16. Mitsuya, H., Weinhold, K. J., Furman, P. A., St. Clair, M. H., Lehrman, S. N., Gallo, R. C., Bolognesi, D., Barry, D. W., Broder, S. *Proc. Natl. Acad. Sci.* **1985**, *20*, 7096.
17. Marqeuz, V. E., Ben-Kasus, T., Barchi, Jr., J. J., Green, K. M., Nicklaus, M. C., Agbaria, R. *J. Am. Chem. Soc*. **2004**, *126*, 543.
18. Jacobson, K. A., Tosh, D. K., Toti, K. S., Ciancetta, A. *Drug Discovery Today*, **2017**, *22*, 1782.
19. Tosh, D. K., Finley, A., Paoletta, S., Moss, S. M., Gao, Z-G., Gizewski, E. T., Auchampach, J. A., Salvemini, D., Jacobson, K. A. *J. Med. Chem.* **2014**, *57*, 9901.
20. Maruoka, H., Barrett, M. O., Ko, H., Tosh, D. K., Melman, A., Burianek, L. E., Balasubramanian, R., Berk, B., Costanzi, S., Harden, T. K., Jacbonson, K. A. *J. Med. Chem*. **2010**, *53*, 4488.
21. Melman, A., Zhong, M., Marquez, V. E., Jacobson, K. A. *J. Org. Chem.* **2008**, *73*, 8085.
22. Toti, K. S., Osborne, D., Ciancetta, A., Boison, D., Jacobson, K. A. *J. Med. Chem*. **2016**, *59*, 6860.
23. Choi, W. J., Park, J. G., Yoo, S. J., Kim, H. O., Moon, H. R., Chun, M. W., Jung, Y. H., Jeong, L. S. *J. Org. Chem*. **2001**, *66*, 6490.
24. Kim, G., Yoon, J., Jarhad, D. B., Shin, Y. S., Majik, M. S., Mulamoottil, V. A., Hou, X., Qu, S., Park, J., Baik, M.-H., Jeong, L. S. *Org. Lett*. **2017**, *19*, 5732.
25. Altmann, K-H., Imwinkelried, R., Kesselring, R., Rihs, G. *Tetrahedron*

Lett. **1994**, *35*, 7625.

26. Oldenziel, O. H., van Leusen, D., van Leusen, M. *J. Org. Chem.*, **1997**, *42*, 3114.
27. Lee, H. W., Nam, S. K., Choi, W. J., Kim, H. O., Jeong, L. S. *J. Org. Chem*. **2011**, *76*, 3557.
28. Lee, J. A., Kim, H. O., Tosh, D. K., Moon, H. R., Kim, S. H., Jeong, L. S. *Org. Lett*. **2006**, *8*, 5081.
29. Jung, M., Offenbacher, G., Retey, J. *Helv. Chim. Acta*, **1983**, *66*, 1915.
30. Lorenz, J. C., Long, L., Yang, Z., Xue, S., Xie, Y., Shi, Y. *J. Org. Chem*. **2004**, *69*, 327.
31. Comin, M. J., Agbaria, R., Ben-Kasus, T., Huleihel, M., Liao, C., Sun, G., Nicklaus, M. C., Deschamps, J. R., Parrish, D. A., Marquez, V. E. *J. Am. Chem. Soc*. **2007**, *129*, 6216.
32. Marquez, V. E., Russ, P., Alonso, R., Siddiqui, M. A., Hernandez, S., George, C., Nicklaus, M. C., Dai, F., Ford, Jr., H. *Helv. Chim. Acta*, **1999**, *82*, 2119.
33. Saneyoshi, H., Deschamps, J. R., Marquez, V. E. *J. Org. Chem.*, **2010**, *75*, 7659.
34. Terrazas, M., Avino, A., Siddiqui, M. A., Marqeuz, V. E., Eritja, R. *Org. Lett.* **2011**, *13*, 2888.
35. Satana, L., Teijeira, M., Uriarte, E. J. *Heterocyclic Chem.*, **1999**, *36*, 293.

Part II.

Southern-Locked C_6 Impact on Leadzyme Self-Cleavage

1. Introduction

Ribozymes are RNA strands that catalyses biochemical transformations. As the name implies, it is a molecule acting like an enzyme. Leadzyme is a ribozyme that cleaves itself in the presence of lead ion,[1] which has a cleaving site at cytidine 6 (Figure 9).[2] Because these self-cleaving ribozymes are known to cleave specific sites, chemically engineered ribozymes getting attention as a potential therapeutic tool.[3] In order to develop ribozymes as a therapeutic tool, it would be essential to understand the nature of it.

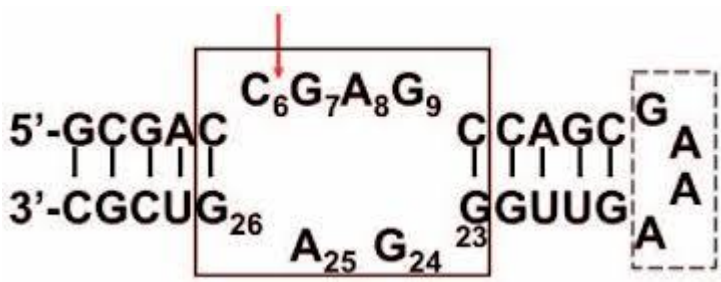


Figure 1. Leadzyme sequence and the cleaving site at C_6.[2]

The RNA self-cleavage is known occur when 2'-OH of the ribose ring is deprotonated, which initiates the nucleophilic attack on the adjacent phosphodiester bond, consequently detaching the neighboring 5'-hydroxyl group, leading to the cleavage of the RNA.[4] In order for the RNA cleavage to occur, the phosphate geometry requires an in-line orientation for an efficient self-cleavage (Figure 10).[5] In this manner, the cytidine 6 of the leadzyme is expected to prefer south conformation over the north for an efficient RNA autohydrolysis.

Hoogstraten and co-workers have shown that replacing cytidine 6 with north-locked cytidine built on a bicyclo[3.1.0]hexane template significantly retards the rate of leadzyme self-cleaving activity.[6] However, the proof of concept will only be valid once the cytidine 6 locked in a southern conformation restores, or accelerate, the rate of leadzyme self-cleavage. For

this purpose, the synthesis of south methanocarba cytidine phosphoramidite is required.

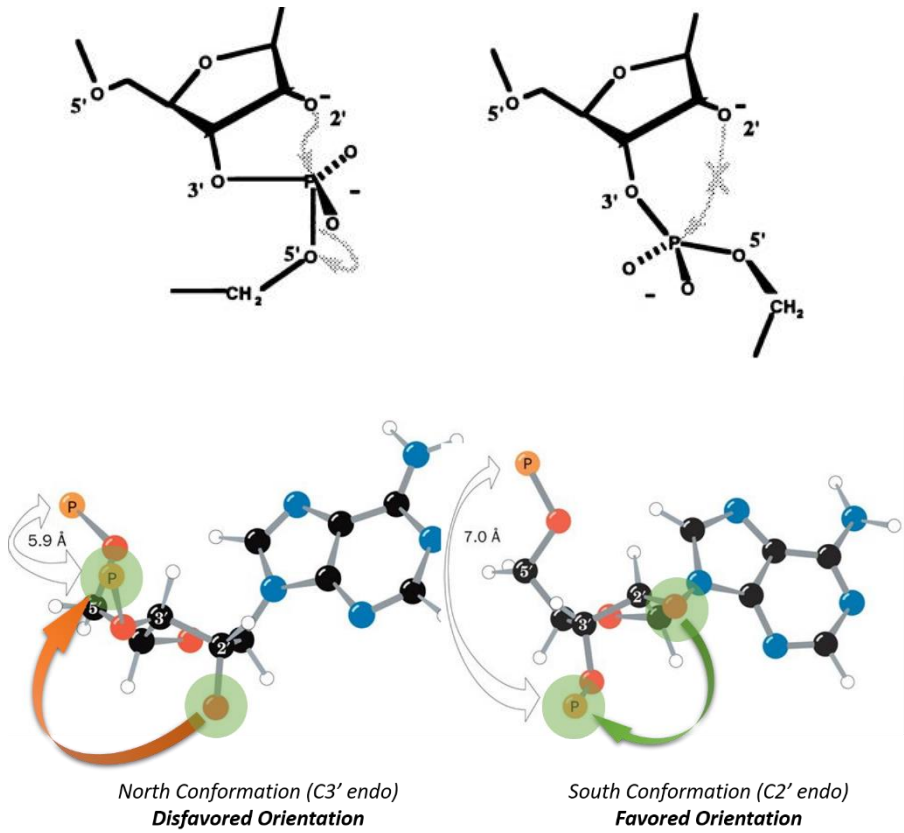


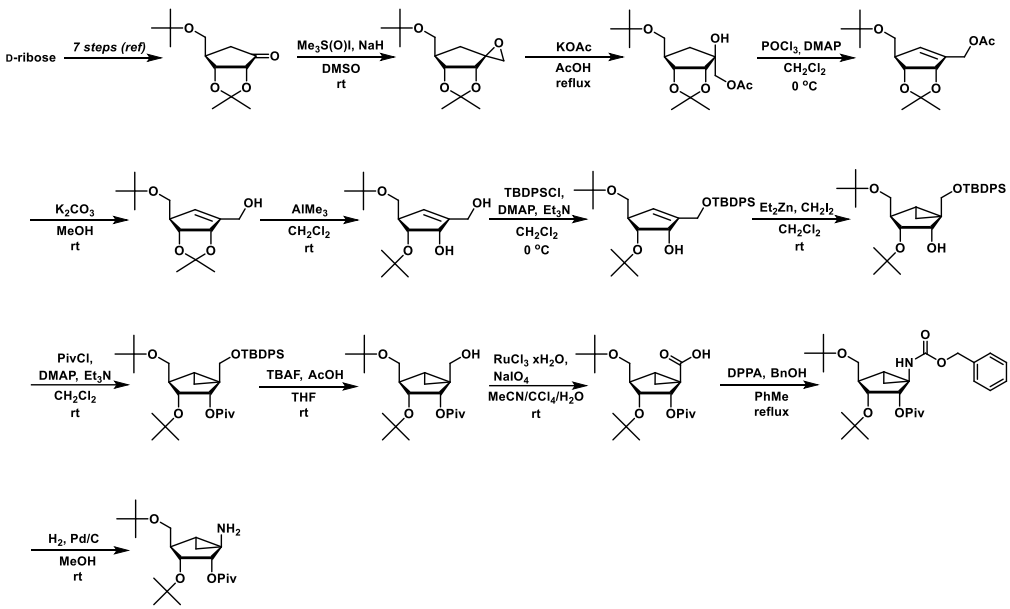
Figure 2. Graphical representation of the "in-line" geometry for efficient RNA hydrolysis. Figure redrawn from the work of Murray, J. B., *et. al*.[5] (top) and expected preferred conformation for efficient self-cleavage (bottom).

2. Synthesis of South Methanocarba Cytidine

2.1 Improving the Synthesis of South Methanocarba Uridine

Because the synthesis of south methanocarba cytidine requires south uridine as the starting point,[7] it was necessary at think point to think about the overall efficiency of the current synthetic approach whether the synthesis is practically achievable. As shown in Scheme 20, 13 steps were

needed to reach the key intermediate, including considerable number of steps spent on simple protection and deprotection protocols. Therefore, an alternative and efficient route to access south restricted template built on a bicyclo[3.1.0]hexane template has been explored.

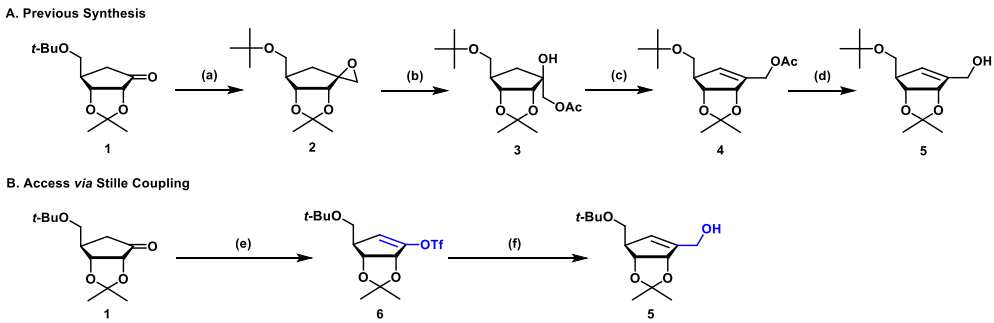


Scheme 1. Synthetic route towards the south bicyclo[3.10]hexane key intermediate

2.1.1. Improvements in Allylic Alcohol Synthesis

Lewis acid-mediated rearrangement of the spiro epoxide by far was the shortest step to the cyclopropanation precursor allylic alcohol **5**, although complete conversion was never achieved. On the other hand, the protocol proposed by Jung *et. al.*[8] was the fastest way to access **5** but in longer steps. Improvements were in need. As a replacement, Stille coupling was applied for efficient installation of the allylic alcohol moiety. The preparation of the hydroxymethyl unit was accomplished by following a reported procedure, and the vinyl triflate was synthesized by trapping the enolate with $PhNTf_2$ as shown in Scheme 2. The protocol was much more efficient than the

previous approaches, providing the allylic alcohol **5** from the carbocyclic template **1** in 72% in 2 steps. A direct formation of the vinyl triflate from 1,4-Michael addition (see Part I.2.1) was carried out to even shorten the steps; however, hydrolysis to the corresponding ketone resulting in **1** was observed. Gram-scale synthesis of allylic alcohol was then conducted by the Stille coupling method.



Scheme 2. Improved synthesis of allylic alcohol 5. Reagents and conditions: (a) $Me_3S(O)I$, NaH, DMSO, 0 ºC to rt, 10 min, 81%; (b) KOAc, AcOH, 120 ºC, 2 h, 84%; (c) $POCl_3$, DMAP, CH_2Cl_2, 0 ºC, 30 min, 80%; (d) K_2CO_3, MeOH, rt, 1 h, 92%; (e) $PhNTf_2$, LiHMDS, THF, -78 ºC to rt, 2 h, 90%; (f) Bu_3SnCH_2OH, $Pd(PPh_3)_4$, LiCl, THF, 65 ºC, 4 h, 80%.

2.1.2. Improvements in Asymmetric Cyclopropanation Reactions

The 5 steps from allylic alcohol, 1) regioselective isopropylidene cleavage; 2) protection of the primary allylic alcohol; 3) Simmons-Smith cyclopropanation reaction; 4) pivaloyl protection; and 5) TBDPS deprotection, were spent on constructing the chiral bicyclo[3.1.0]hexane template. If a direct asymmetric cyclopropanation can be achieved at this stage, a significant improvement can be expected.

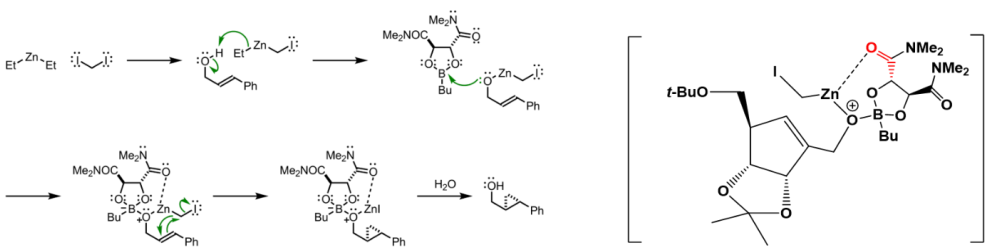
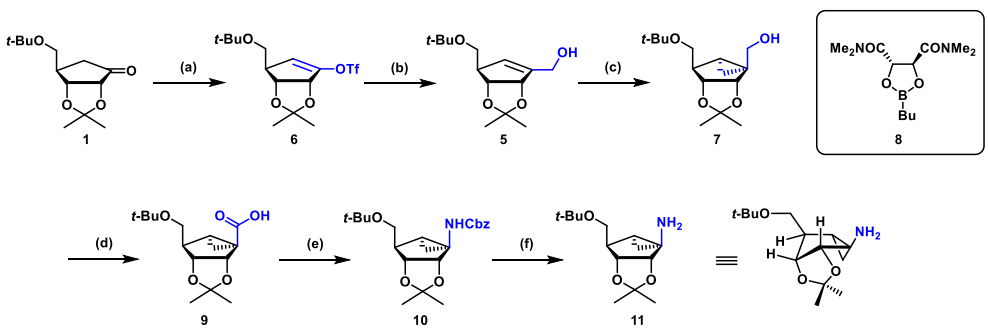


Figure 3. Mechanism of Charette's asymmetric cyclopropanation reaction (left)[10] and expected transition state (right).

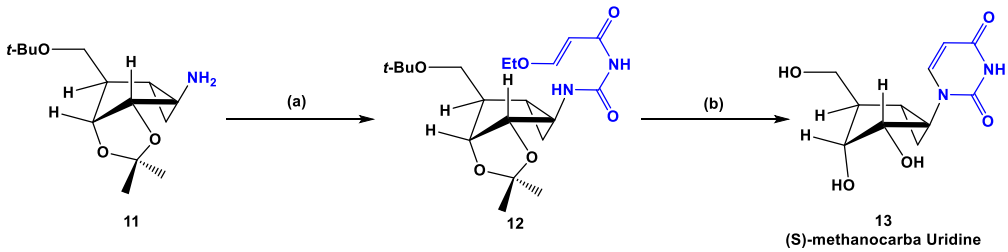
Several options were considered, among which Charette's chiral borane ligand was chosen as the top priority.[9] The priority was given based on commercial availability and substrate scope, which Charette's reagent satisfied both. The chiral auxiliary-mediated cyclopropanation was a great success, providing the product exclusively with the desired stereochemistry. The only drawback was that it is used as a chiral auxiliary that requires molar equivalent of the reagent, rather than in catalytic amount, for asymmetric induction. However, the preparation of the reagent is convenient enough for gram scale reactions thus no further efforts were invested in improving efficiency.

2.1.3. Efficient Synthesis of South Methanocarba Uridine

With considerable improvements, an efficient synthetic route to the key intermediate **11** has been established as shown in Scheme 3. The synthesis of south methanocarba uridine followed the conventional base build-up approach analogous to the previously reported method (Scheme 4. See Part I.2.3.2) to furnish the south methanocarba uridine in an overall yield of 7% in 15 steps from D-ribose. The synthetic approach proposed in this study if significantly superior than previously reported methods.

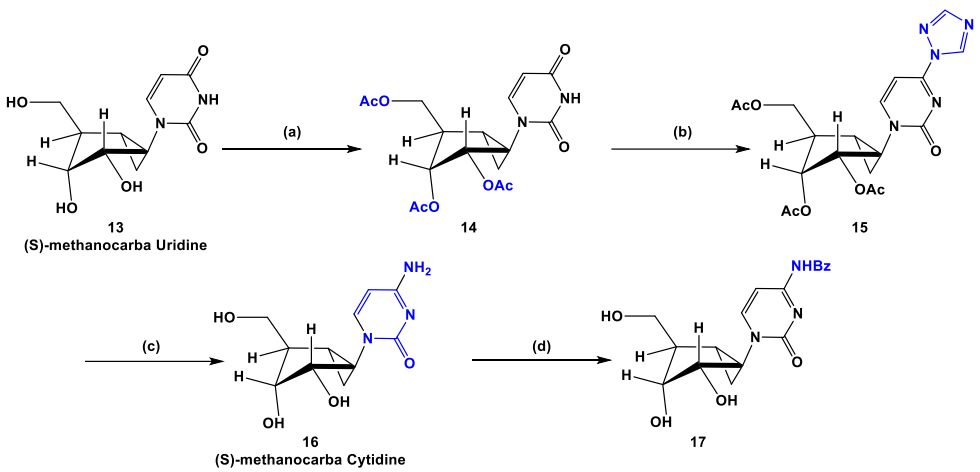


Scheme 3. Efficient Synthesis of Bicyclo[3.1.0]hexane Template. Reagents and conditions: (a) LiHMDS, $PhNTf_2$, THF, -78 °C to rt, 2 h, 90%; (b) Bu_3SnCH_2OH, $Pd(PPh_3)_4$, LiCl, THF, 65 °C, 4 h, 80%; (c) **8**, 1,2-DME, Et_2Zn, CH_2I_2, -15 °C to rt, 15 h, 83%; (d) $RuCl_3 \cdot xH_2O$, $NaIO_4$, CCl_4/MeCN/H_2O, 0 °C, 4 h, 70%; (e) i) DPPA, Et_3N, ii) BnOH, PhMe 110 °C, 5 h, 81%; (f) H_2, Pd/C, MeOH, rt, 16 h, 99%.



Scheme 4. Synthesis of south methanocarba uridine. Reagents and conditions: (a) (*E*)-3-ethoxyacryloyl isocyanate, CH_2Cl_2, 0 °C to rt, 2 h, 82%; (b) HCl, THF, 80 °C, 2 h, 72%.

2.2 Synthesis of South Methanocarba *N*-Benzoyl Cytidine



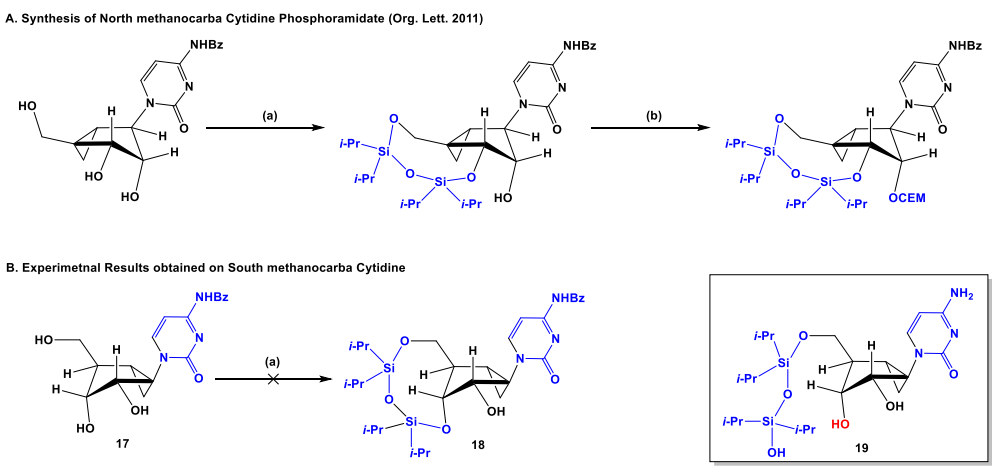
Scheme 5. Synthesis of South methanocarba *N*-benzoyl cytidine. Reagents and conditions: (a) Ac_2O, DMAP, Et_3N, CH_3CN, rt, 24 h, 86%; (b) 1,2,4-triazole, $POCl_3$, CH_3CN, 0 °C to rt, 6 h, 80%; (c) i) NH_4OH, 16 h, ii) NH_3/MeOH, 16 h, 71% for 2 steps; (d) benzoic anhydride, DMF, rt, 24 h, 76%.

The preparation of south methanocarba cytidine followed the procedure reported for its north analogue,[7] which is presented in Scheme 5. South methanocarba uridine **13** was peracetylated to give **14**, followed by substitution reaction with 1,2,4-triazole afforded **15** in good yields. The crude residue was treated with ammonium hydroxide, followed by methanolic ammonia to afford south methanocarba cytidine **16** in three steps, with partial product reverting to south methanocarba uridine **13**. The amount returning to **13** varied upon batch to batch. In the north analogue,[7] the *N*-

benzoylation was achieved by temporarily masking the free hydroxyl groups with trimethylsilyl (TMS) groups; however, a selective protection of the amine was achieved using benzoic anhydride in dry DMF.

2.3 Synthesis of South Methanocarba Cytidine Phosphoramidite Precursor

The preparation of nucleoside phosphoramidite for oligonucleotide synthesis requires selective manipulations of 2', 3', and 5'-hydroxyl groups.[7] This is usually achieved by 1) protection of the amino group of cytosine; 2) regioselective protection of 5'- and 3'-hydroxyl groups with TIPDS (tetraisopropyldisiloxane); 3) protection of 2'-hydroxyl group with TOM (triisopropyloxymethyl) or CEM (cyanoethoxymethyl); 4) deprotection of the TIPDS group; 4) regioselective protection of 5'-primary hydroxyl group with an acid labile group, most commonly DMT (dimethoxytrityl); 5) phosphoramidate attachment to 3'-hydroxyl group. The preparation of north methanocarba cytidine analogue was prepared by following this common procedure; thus the synthesis of the south methanocarba cytidine phosphoramidite was planned as shown in Scheme 6.



Scheme 6. Synthesis of the reported north methanocarba cytidine phorphoramidate synthesis (A) and its application on the south analogue (B).

Reagents and conditions: (a) 1,3-dichlorotetraisopropyldisiloxane, imidazole, pyridine, 83%; (b) i) MeSCEM/THF, ii) CF_3SO_3H, NIS, - 45 ºC, iii) Et_3N, $Na_2S_2O_4$, 73%.

With south methanocarba *N*-benzoyl cytidine **17** in hand, regioselective protection of 5'- and 3'-hydroxyl groups with TIPDS was carried out. However, surprisingly the product obtained was 5'-hydroxy, mono protected product **19** rather than the desired product **18**. A plausible explanation for this phenomenon involves physical aspects, due to the distance from 5'-*O* and 3'-*O*s in south being much further than the north analogue (Figure 4).

In other words, the TIPDS seemed to be simply not long enough to reach both ends. Ethylene-bridged, as a replacement of the oxygen linker, disilylating agent STABSE (1,2-bis(dichloromethylsilyl)ethane) was used as an alternative expecting that the carbon homologation would reach both ends, however the result also resulted in a mono protected product.

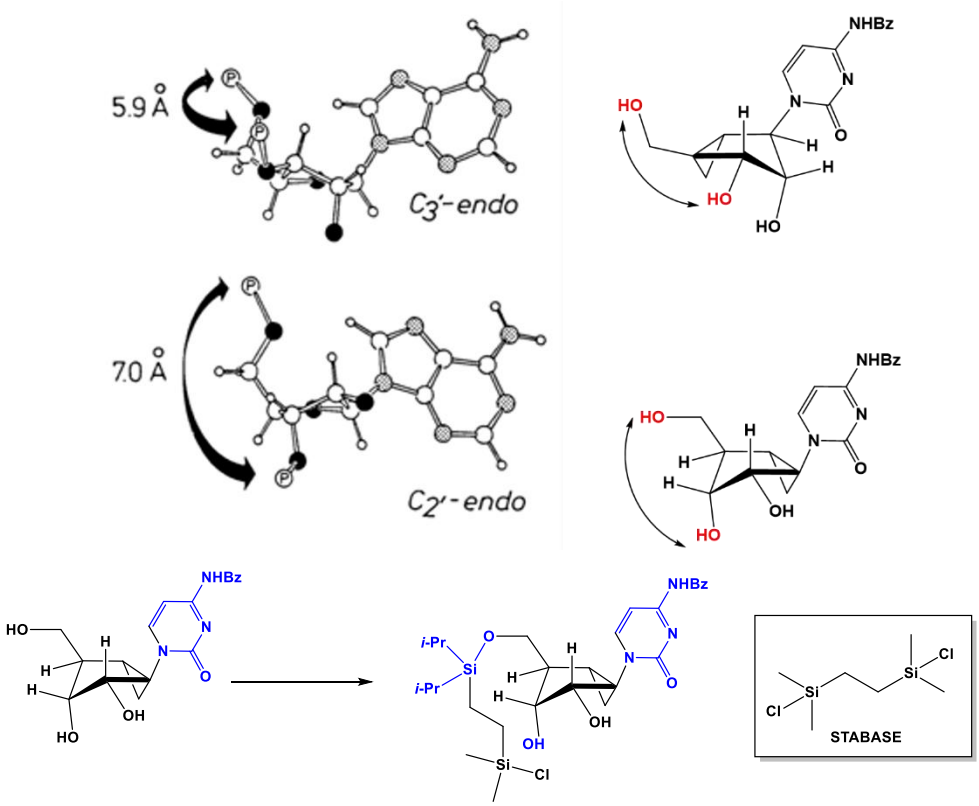
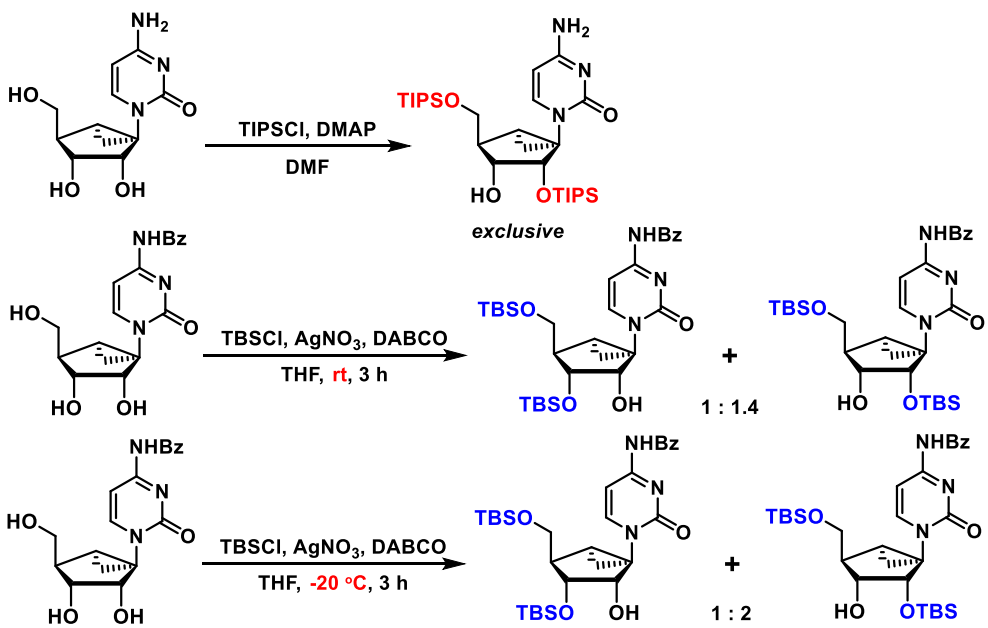


Figure 4. A plausible explanation for the formation of **19** includes their physical aspects. The distance may be simply too far for the reagent to reach

both ends. However, employing STABASE was also ineffective.

An alternative method to selectively protect the 3'-hydroxyl group in the presence of 2'-hydroxyl was needed. Hypothesis based on steric factor was proposed: sterically bulky protecting group such as TIPS would prefer the 3'-hydroxyl over the 2'-hydroxyl due to the steric repulsion caused by the neighboring cyclopropyl group, however in fact this reaction resulted in a 2'-selective silylation. This result is perhaps due to the 2'-hydroxyl group being placed in an equatorial axis and the 3'-hydroxyl in an axial orientation. That is, the steric influence caused by the cyclopropyl group is in fact greater in the case of 3'-hydroxyl group.

When a sterically less bulky TBS group was employed, the protection occurred as a mixture, the 2'-hydroxyl protection being the major product in lower temperature. Near 1:1 ratio was obtained at room temperature. The two were nearly identical in R_fs, making the separation extremely difficult, but the separation was possible and the desired 5'-*O*, 3'-*O*-disilylated south methanocarba *N*-benzoyl cytidine was obtained.



Scheme 7. Preparation of the 5'-*O*, 3'-*O*-disilylated South methanocarba *N*-

Bz cytidine as a phosphoramidate precursor for solid-phase oligonucleotide synthesis

3. Conclusion

In order to synthesize south conformation restricted cytidine, an improved synthesis of south conformation-locked uridine built on a bicyclo[3.1.0]hexane template was achieved via Stille coupling, Charette asymmetric cycopropanation and base build up reactions as key steps. The synthetic method proposed in this study is by far the most efficient method providing south methanocarba uridine in 7% overall yield in 15 steps from D-ribose. The south methanocarba uridine was successfully converted to cytidine analogue, which in turn underwent 5'-*O*, 3'-*O* protection to furnish the phosphoramidate precursor for solid-phase oligonucleotide synthesis.

4. Experimental Section

General Methods

Proton (^{1}H), carbon (^{13}C) NMR, spectra were recorded on a Bruker AV 400 (400/100 MHz), Bruker AMX 500 (500/125 MHz), or Jeol JNM-ECA600 (600/150 MHz). Chemical shifts are given in parts per million (ppm) (δ) relative to the solvent peak. Coupling constants (J) are reported as Hertz (Hz). High resolution mass spectra (HRMS) measurements were performed on a Thermo LCQ XP instrument. Optical rotations were determined on Jasco III in appropriate solvent. UV spectra were obtained on U-3000 made by Hitachi in methanol or water. Melting points were determined on a Buchan B-540 instrument and are uncorrected. Reactions were checked by thin layer chromatography (Kieselgel 60 F254, Merck). Spots were visualized under UV light (254 nm), or by colorizing and charring with a *p*-anisaldehyde solution or a phosphomolybdic acid solution. The crude compounds were purified by silica gel column chromatography (Kieselgel 60, 70-230 mesh, Merck). All the anhydrous solvents were

redistilled over CaH_2, P_2O_5, or sodium/benzophenone before the reaction. All reactions were performed under nitrogen atmosphere and anhydrous solvents unless specified otherwise.

((3a*R*,4*R*,6a*S*)-4-(*tert*-butoxymethyl)-2,2-dimethyl-3a,6a-dihydro-4*H*-cyclopenta[*d*][1,3]-dioxol-6-yl)methanol (5)

To a stirred solution of 6 (1.17 g, 3.13 mmol) in dry THF (16 mL) was sequentially added LiCl (265 mg, 6.26 mmol), Bu_3SnCH_2OH (2.01 g, 6.26 mmol) and $Pd(PPh_3)_4$ (358 mg, 0.31 mmol) and the resulting reaction mixture was heated to 70 ºC under nitrogen atmosphere for 4 hours. The reaction was cooled to 0 ºC and aqueous sodium hydroxide was added and left stir at room temperature overnight. The aqueous phase was then extracted with ethyl acetate thrice, and the combined organic layer was washed with brine, dried over anhydrous magnesium sulfate and concentrated in vacuo. The crude residue was purified by silica gel column chromatography to afford the title compound **5** (642 mg, 2.50 mmol) as a colorless oil.

(3a*R*,4*R*,6a*R*)-4-(*tert*-butoxymethyl)-2,2-dimethyl-3a,6a-dihydro-4*H*-cyclopenta[*d*][1,3]-dioxol-6-yl trifluoromethanesulfonate (6)

To a stirred solution of 1 (1.05 g, 4.33 mmol) in dry tetrahydrofuran (14 mL) was added LiHMDS 1.0M solution in THF (5.63 mL, 5.63 mmol) dropwise at -78 ºC, followed by N-phenyl-bis(trifluoromethanesulfonimide (1.86 g, 5.2 mmol) and the resulting reaction mixture was stirred at -78 oC for 30 minutes and stirred at room temperature for an additional 30 minutes. The reaction was cooled to 0 ºC, and was quenched with saturated aqueous ammonium chloride solution and poured into water (30 mL). The aqueous layer was extracted with ethyl acetate twice, and the combined organic layer was washed with brine, dried over anhydrous magnesium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel column

chromatography to afford the title compound **6** (1.46 g, 90%) as a colorless oil.

((3a*S*,3b*R*,4a*S*,5*R*,5a*R*)-5-(*tert*-butoxymethyl)-2,2-dimethyltetrahydrocyclopropa[-3,4]cyclopenta[1,2-*d*][1,3]dioxol-3b(3a*H*)-yl)methanol (7)

To a stirred solution of **5** (180 mg, 0.70 mmol) in dry dichloromethane (10 mL0 was added 1,2-dimethoxyethane (0.15 mL, 1.40 mmol), Charette's chiral borane ligand (246 mg, 0.91 mmol), diethylzinc (1.40 mL, 1.40 mmol, 1.0M in hexane solution), followed by diiodomethane (0.17 mL, 2.80 mmol) at – 15 oC under nitrogen atmosphere and the resulting reaction mixture was stirred at room temperature for 4 hours. An additional equivalent of 1,2-dimethoxyethane, diethylzinc and diiodomethane was added and the resulting reaction mixture was stirred at room temperature overnight. The reaction was quenched by the addition of saturated aqueous ammonium chloride and poured into water. The layers were separated, and the aqueous layer was extracted with dichloromethane twice. The combined organic layer was washed with brine, dried over anhydrous magnesium sulfate and concentrated in vacuo. The crude residue was purified by silica gel column chromatography to afford the title compound **7** (157mg, 83%) as a colourless oil.

(3a*S*,3b*S*,4a*S*,5*R*,5a*R*)-5-(*tert*-butoxymethyl)-2,2-dimethyltetrahydrocyclopropa[3,4]cycl-openta[1,2-*d*][1,3]dioxole-3b(3a*H*)-carboxylic acid (9)

To a stirred solution of **7** (157 mg, 0.58 mmol) in MeCN/CCl_4/H_2O (4.5 mL, 2:2:3 v/v) was added ruthenium chloride hydrate (12 mg ,0.06 mmol), followed by sodium periodiate (248 mg, 1.16 mmol) and the resulting reaction mixture was stirred at 0 ºC for 4 hours. The reaction was filtered through a pad of Celite, and the resulting mixture was concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography eluting hexane/ethyl acetate (1:1) with 1 mL of acetic acid in every 100 mL of

eluting solvents to afford the title compound **9** (115 mg, 70%) as a colorless oil.

Benzyl ((3a*S*,3b*S*,4a*S*,5*R*,5a*R*)-5-(*tert*-butoxymethyl)-2,2-dimethyltetrahydrocyclopropa-[3,4]cyclopenta[1,2-d][1,3]dioxol-3b(3aH)-yl)carbamate (10)

To a stirred solution of **9** (115 mg, 0.41 mmol) in dry toluene (7 mL) was added triethylamine (0.17 mL, 0.49 mmol) followed by DPPA (0.11 mL, 0.49 mmol) at 0 ºC and the resulting solution was stirred under nitrogen atmosphere for 30 minutes. To the solution was then added benzyl alcohol (0.06 mL, 0.62 mmol) and the resulting reaction mixture was heated to reflux and stirred for 4 hours. The reaction was cooled room temperature and the volatiles were removed in vacuo. The crude residue was purified by silica gel column chromatography to afford the title compound **43** (129 mg, 81%) as a colorless oil.

(3a*S*,3b*S*,4a*S*,5*R*,5a*R*)-5-(*tert*-butoxymethyl)-2,2-dimethyltetrahydrocyclopropa[3,4]cyc-lopenta[1,2-*d*][1,3]dioxol-3b(3a*H*)-amine (11)

To a stirred solution of **10** (129 mg, 0.33 mmol) in methanol (10 mL) was added palladium on activated carbon (4 mg, 0.03 mmol) and the resulting reaction mixture was stirred under an atmosphere of hydrogen for 16 hours. The crude residue was filtered through a pad of Celite, and the volatiles were removed in vacuo. The crude residue **11** (83 mg, 99%) was used directly without any further purification.

(*E*)-*N*-(((3a*S*,3b*R*,4a*S*,5*R*,5a*R*)-5-(*tert*-butoxymethyl)-2,2-dimethyltetrahydrocyclopropa-[3,4]cyclopenta[1,2-*d*][1,3]dioxol-3b(3a*H*)-yl)carbam-

oyl)-3-ethoxyacrylamide (12)

To a stirred solution of **11** (83 mg, 0.33 mmol) in dry dichloromethane (5 mL) was dropwise added freshly prepared (*E*)-3-ethoxyacryloyl isocyanate 0.4M solution in toluene (1.65 mL, 0.66 mmol) at 0 ºC under nitrogen atmosphere and the resulting reaction mixture was stirred at room temperature for 4 hours. The volatiles were removed in vacuo and the crude residue was purified by silica gel column chromatography to afford the title compound **12** (107 mg, 82%) as a colorless oil.

1-((1*S*,2*S*,3*R*,4*R*,5*S*)-2,3-dihydroxy-4-(hydroxymethyl)bicyclo[3.1.0]hex-an-1-yl)pyramid-ine-2,4(1*H*,3*H*)-dione (13)

Compound **12** (107 mg, 0.27 mmol) was dissolved in a small amount of tetrahydrofuran (0.5 mL) and 2*N* HCl solution (3 mL) was added. The resulting solution was then heated to 80 ºC and stirred for 4 hours. The reaction was cooled to room temperature and the volatiles were removed *in vacuo*. The crude residue was purified by silica gel column chromatography to afford the title compound **13** (49 mg, 72%) as a white solid.

5. References

1. Pan, T., Uhlenbeck. *Biochemistry*, **1992**, *31*, 3887.
2 Julien, K. R., Sumita, M., Chen, P-H., Laird-Offringa, I. A., Hoogstraten, C. G. *RNA*, **2008**, *14*, 1632.
3. Kashiani-Sabt., M. *J. Investig. Dermatol. Symp. Proc*. **2002**, *7*, 76.
4. Donalt, V. Judith, V. *Biochemistry* (4 ed.), Wiley & Sons, **2011**.
5. Murray, J. B., Terwey, D. P., Maloney, L., Karpeisky, A., Usman, N., Beigelman, L., Scott, W. G. Cell, **1998**, *92*, 665.
6. White, N. A., Sumita, M., Marqeuz, V. E., Hoogstraten, C. G. *RNA*, **2018**, *24*, 1542.

7. Terrazas, M., Avino, A., Siddiqui, M. A., Marqeuz, V. E., Eritja, R. *Org. Lett.* **2011**, *13*, 2888.
8. Jung, M., Offenbacher, G., Retey, J. *Helv. Chim. Acta*, **1983**, *66*, 1915.
9. Charette, A. B., Juteau, H., Lebel, H., Molinaro, C. *J. Am. Chem. Soc.*, **1998**, *120*, 11943.
10. Charette Asymmetric Cyclopropanation. In The Organic Synthesis Archive. https://www.synarchive.com/named-reactions/Charette-Asymmetric-Cyclopropanation. (Accessed June 28, 2019)

Part III.

An Alternative and Efficient Synthesis of MLN4924, A Selective NEDD8-Activating Enzyme Inhibitor

1. Introduction

Although U.S. alone has spent over $200 billion on cancer research since 1971,[1] statistic has shown that the rate of cancer death had only declined by 5% from 1950 to 2005,[2] proving that the current progress in drug development is yet far from defeating cancer. The Food and Drug Administration (FDA) recently announced that 59 drugs were approved in 2018,[3a] among which 27% were designated as anticancer drugs.[3b] Although recent breakthroughs in immunotherapies and antibody research have brought attention to biologics as a promising next generation therapy,[4] the fact that more than half of the approved drugs in 2018 are small molecules (38 out of 59, accounting for 66% of the total approved drugs in 2018),[3c] denotes that the pharmaceutical research still largely relies on classical structure-based approach. Numerous targets for cancer therapy have been discovered through intensive research in the past years.[5] In one of these studies NEDD8-activating enzyme (NAE) was introduced as a potential fresh target for anticancer therapeutics.[6] NAE, which exists as a heterodimeric molecule of amyloid beta precursor protein-binding protein 1 (APPBP1) and ubiquitin-like modifier activating enzyme 3 (UBA3),[7] initiates the catalytic cycle that forms a NEDD8-AMP intermediate by binding with an ATP and NEDD8. The NEDD8-AMP intermediate formed in this process then binds to the adenylation domain of the NAE, transferring its NEDD8 to the catalytic cysteine of the UBA3 by forming a thioester linkage. After repeating a similar cycle with a second NEDD8-AMP adduct, an NAE with two activated NEDD8 molecule is formed.[7] This NAE with two NEDD8 is then capable of activating cullin-RING ligases (CRLs), which are known to have significant role in diverse biological pathways including proteasome-mediated protein degradation.[8] This process is known as neddylation.

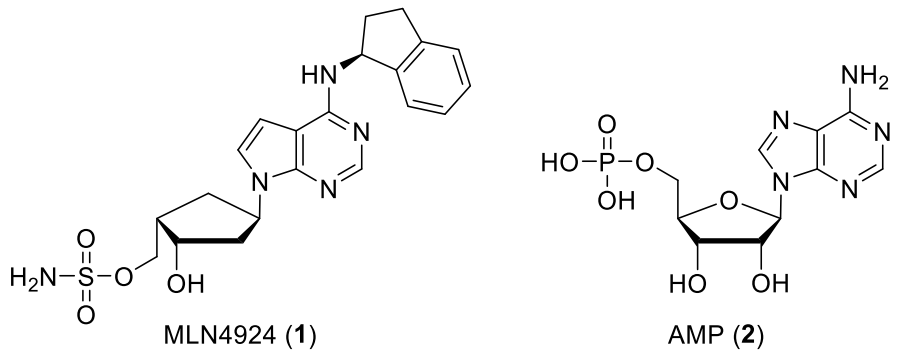


Figure 1. The structure of MLN4924 (**1**) as an AMP (**2**) mimetic.

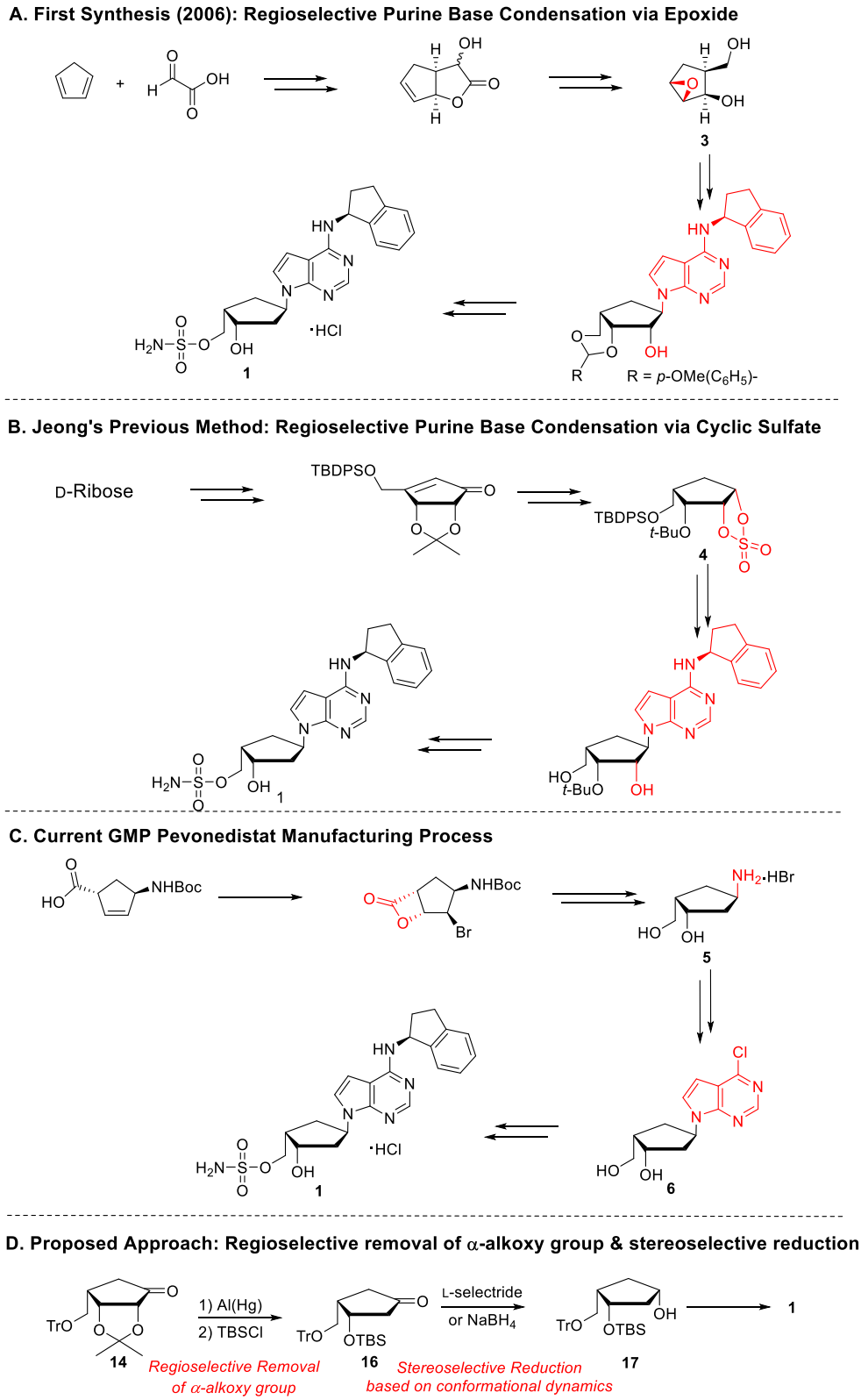
MLN4924 (**1**), also known as pevonedistat, is a small molecule that is designed to selectively inhibit NEDD8-activating enzyme by acting as adenosine monophosphate mimetic (AMP, **2**) (Figure 1).[6] The catalytic activity of NAE produces a stable covalent adduct of NEDD8 with MLN4924 in the catalytic pocket of UBA3, thereby forming a NEDD8-MLN4924 adduct. This non-natural adduct is not capable of activating the CRLs, leading to CRL-mediated protein turnover and deregulations in the S-phase of DNA synthesis thereby inducing apoptosis in cancer cells.[6]

MLN4924 first entered the clinical trial as a treatment for acute myeloid leukemia and myelodysplastic syndrome,[9] however, its enormous potential for therapeutic use is still being explored and their findings for possible future applications are actively being reported to date.[10]

Despite its interesting biological activity, only a few syntheses of **1** have been reported in literature.[11-14] Scheme 1 describes the comparison between previous and current syntheses of **1**. The first synthesis of **1** described in U.S. patent began with cyclopentadiene, from which the synthesis of **1** was achieved by employing enzymatic resolution and regioselective purine base condensation via the epoxide **3** as the glycosyl donor.[11] Another elegant method of **1** was achieved from D-ribose using stereoselective reduction, regioselective cleavage of an isopropylidene moiety, and the regioselective

purine base displacement to cyclic sulfate **4** as key steps.[12] The current GMP production of MLN4924 is accomplished via the nucleobase build-up approach of chiral amino diol **5**.[13]

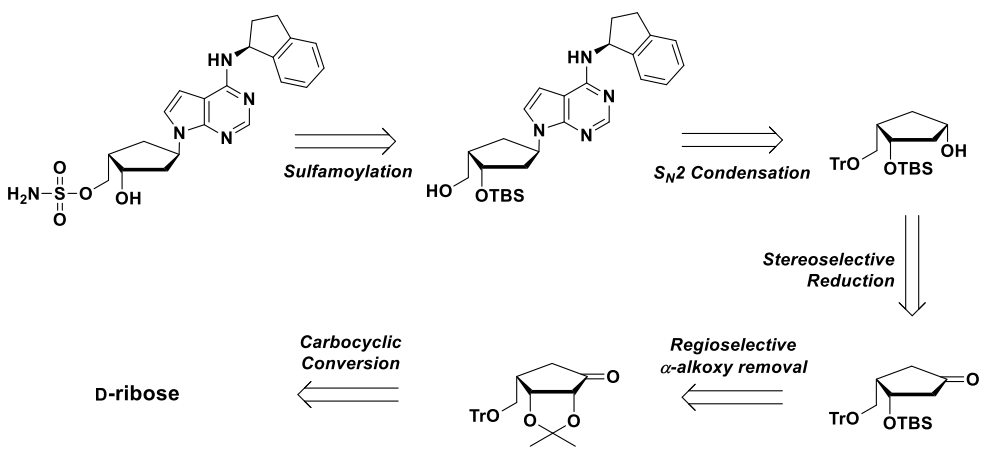
Thus, developing a reliable method that can easily produce structural analogues of **1** was desirable to further explore its potential as a biologically privileged template. While the current GMP production method[13] produces **1** most efficiently to date, yet the chiral amino diol **5** being the key intermediate imposes significant challenge in structural modifications. Moreover, because of the preinstalled stereochemistry of the amine group, a linear synthetic approach in installing the nucleobase moiety is inevitable, decreasing the overall efficiency of the synthesis. The chiral aminoindane substitution reaction of **6** was particularly another struggle as it requires harsh conditions.[14] The current manufacturing process relies on pressurized atmosphere at elevated temperature (80-120 psi at 130 ^{o}C),[14] which was also incompatible with our given circumstance.



Scheme 1. Comparison between previous and present syntheses of **1**

The other reported methods were also difficult to utilize for structural diversification. Barton-McCombie deoxygenation, a two-step sequence that has been most widely used in removing 2'-hydroxy group in nucleosides,[15] including the original patent claim[11] and the method reported by Jeong *et al.*[12] for example, successfully removes the 2'-hydroxyl group; however, because it is a radical-based reaction performed at the late stage of the synthesis, it prevents the introduction of radical-labile functional groups to **1**. Furthermore, because the 2'-hydroxyl group is removed at the late stage of the synthesis, it is rather difficult to functionalize the 2'-position compared to the accessibility in its sugar state.

In order to overcome these drawbacks, a convergent synthetic route via versatile sugar intermediate **16,** by employing regioselective α-alkoxy removal as the key step, is designed.



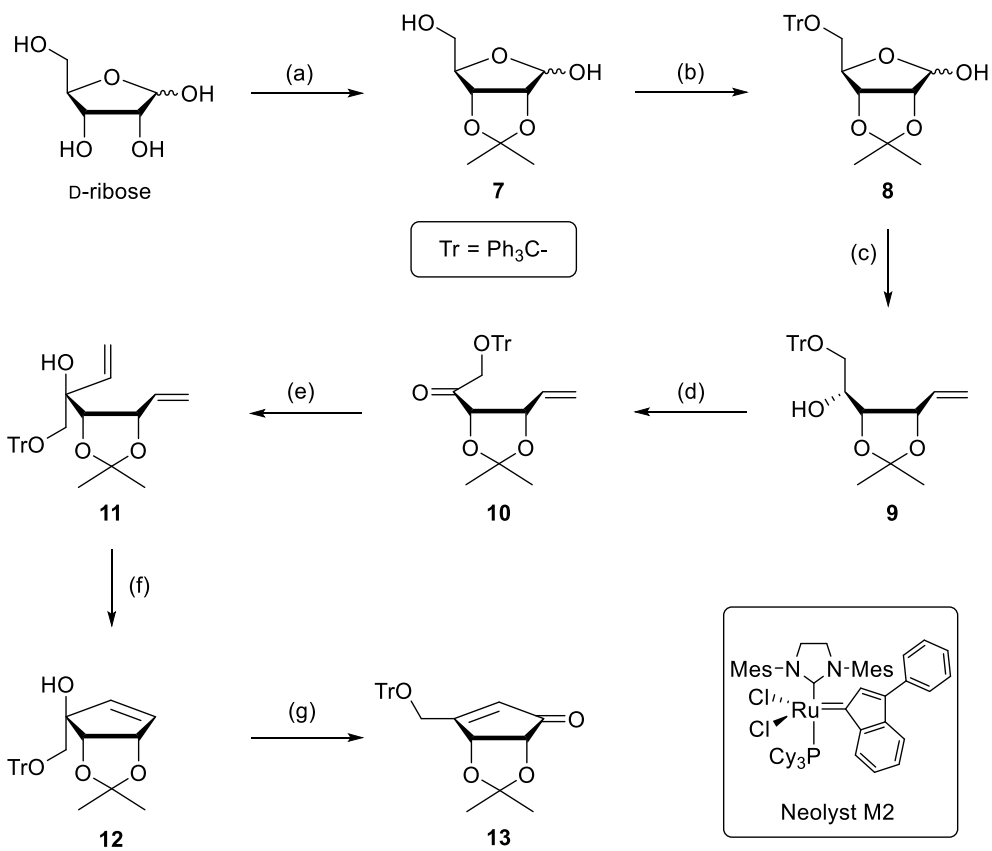
Scheme 2. Retrosynthetic analysis of target compound **1**.

The sulfamoylation was designed to take place at the last stage of the synthesis as it is unstable in many chemical transformations. Introduction of the characteristic MLN4924 nucleobase moiety is expected to be installed in an S_N2 fashion, while the glycosyl donor can be obtained from stereoselective reduction of its ketone. The key deoxygenation is expected to be achieved in a radical-based manner, either by aluminum amalgam or samarium(II) iodide. The conversion of D-ribose to its carbocyclic template

is known.

2. Results and Discussion

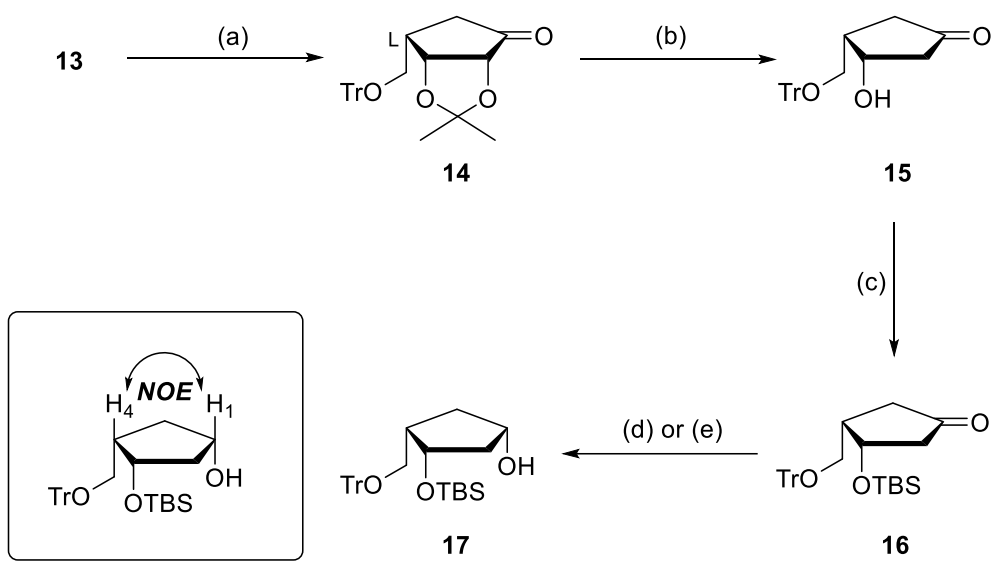
Synthesis of MLN4924 began with converting a naturally abundant sugar moiety into a carbocyclic template by following a reported procedure[16] with minor modifications (Scheme 3), which primarily focused on reducing time and effort spent in purification processes as well as replacing costly reagent to improve the overall efficiency of the synthesis.



Scheme 3. Preparation of carbocyclic sugar **13**. ***Reagents and conditions***: (a) *c*-H_2SO_4, acetone, rt, 2.5 h; (b) trityl chloride, pyridine, rt, 16 h; (c) CH_3PPh_3Br, KO*t*-Bu, THF, rt, 6 h; (d) $(COCl)_2$, DMSO, CH_2Cl_2, -78 °C to rt, 1 h; (e) vinylmangesium bromide, THF, -78 °C, 3.5 h, 72% for 5 steps; (f) 0.02 mol% Neolyst M2, PhMe, rt, 2 d; (g) PDC, 4Å mol. sieves, CH_2Cl_2, rt, 18 h, 63% for 2 steps.

Acetonide protection of D-ribose with catalytic H_2SO_4 in acetone afforded

7, of which after neutralization with solid sodium bicarbonate was pure enough to be used in the subsequent step with a simple filtration work up. The trityl group was selected as the protecting group for the primary hydroxyl group as we had plans for employing silyl protecting group in later stage of the synthesis which needed selective deprotection. Thus, protection of the primary hydroxyl group with trityl chloride yielded **8** and after a simple aqueous work up, **8** underwent Wittig reaction to give **9**. Swern oxidation of **9**, without silica gel column purification, successfully produced ketone **10**, in turn afforded diene **11** after a Grignard reaction with vinylmagnesium bromide. Silica gel column purification was done at this stage to prepare the ring-closing metathesis precursor **11** in 72% yield over 5 steps. Grubbs' 2nd generation catalyst which has been employed in the reported procedure[16] was replaced with Neolyst M2, as the latter was more economically feasible, yet provided the desired cyclized product **12**, which without purification, underwent the oxidative rearrangement with PDC to afford **13** in 63% yield.



Scheme 4. Access to versatile sugar moiety **16** and glycosyl donor **17.** ***Reagents and conditions:*** (a) H_2, Pd/C, Na_2CO_3, EtOAc, rt, 12 h; (b) Al(Hg), THF/H_2O (8:1), rt, 6 h, 81% for 2 steps; (c) TBSCl, imidazole, DMF, rt, 12 h, 88%; (d) L-selectride, THF, -78 ºC, 10 min, 89%; (e) $NaBH_4$,

MeOH, 0 ºC, 10 min, 91%.

Upon securing the carbocyclic sugar, the focus shifted to the preparation of the versatile intermediate **16** as shown in Scheme 4. The installation of 4-L-configuration was accomplished with palladium-mediated hydrogenation of **13** by relying on facial discriminating factors. Basic medium induced by sodium carbonate was necessary in this hydrogenation reaction as deprotection of the trityl group was observed in such condition without it.[17] The hydrogenated compound **14** was directly used after a simple filtration.

The initial approach in achieving the key regioselective α-alkoxy removal of the isopropylidene group involved samarium(II) iodide as the reductant with ethylene glycol as the proton source;[18] however the reaction did not proceed in this given condition. It was thought that this might be a result of the reaction taking the samarium(III) enolate pathway, as proposed by Molander *et al*.,[19] where the isopropylidene group in this case was not sufficient enough to act as a leaving group. Thus, it was believed that a radical cascade initiated by a single electron transfer process would be more suitable for our system. An attempt with aluminium amalgam as the choice of reductant in THF/H_2O (8:1) as the solvent,[20] cleanly provided alcohol **15** in an excellent yield of 81% in 2 steps. The alcohol **15** was then protected with TBS to give the versatile intermediate cyclopentanone **16**.

Although 2-deoxyketone **16** does not have the privilege of the stereoselective reduction induced by the α-substituent, conformational analysis, illustrated in Figure 2 appears to guarantee the stereoselective reduction with desired stereochemistry. It was hypothesized that the conformational equilibrium would lean towards the molecular shape that keeps the interaction of the two bulky groups, the 4-trityloxymethyl and the 3-OTBS, to a minimum extent; therefore, adopting a half-chair conformation as the predominant conformation over the envelope form. By adopting the half-chair conformation, the pseudo-axial substituent is now placed in nearly an axial orientation with regard to ketone bringing the

proximity of the two groups much closer, thereby imposing greater influence. Furthermore, the two substituents, trityloxymethyl and the TBS, both causing severe steric repulsions would force each other to position the pseudoaxial substituent close to a *syn*-orientation with respect to the cyclopentanone core which blocks incoming nucleophiles approaching from the α-face.

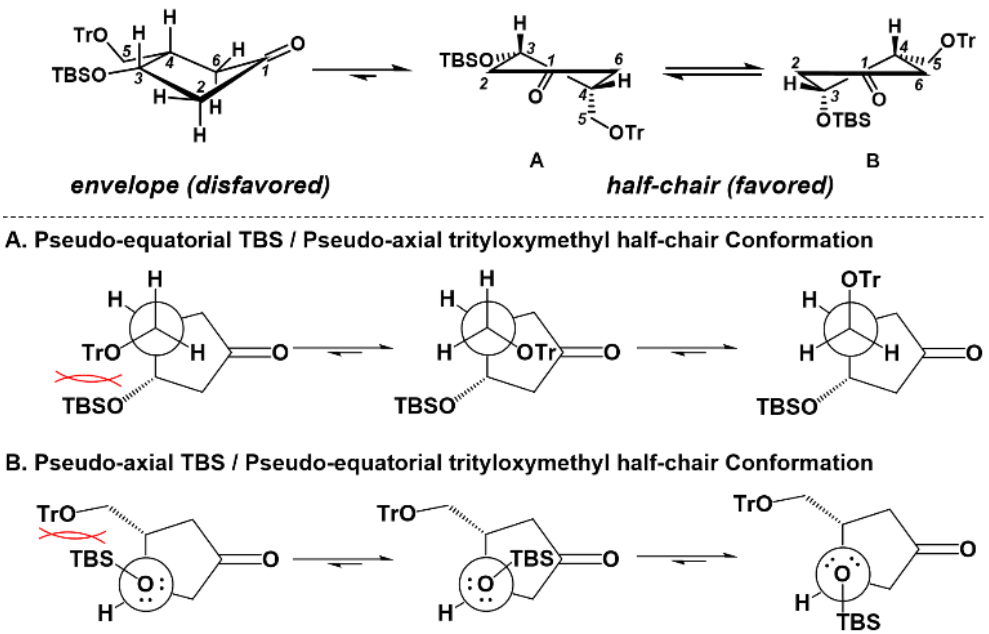
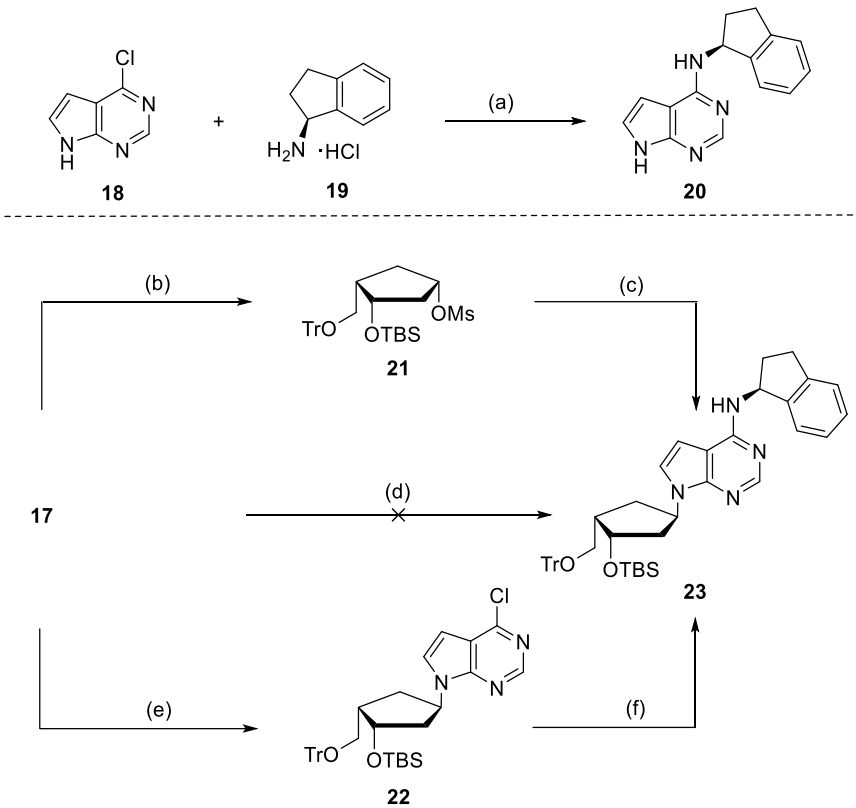


Figure 2. Conformational dynamics of cyclopentanone **16**.

According to the analysis, it was also believed that the size of the reducing agent has no significant effects on stereoselective reduction in this system as the substituents covering the α-face would already be large enough to determine the stereoselectivity. As expected, stereoselective reduction was achieved to afford the desired α-hydroxyl product **17** as the single stereoisomer with excellent yield, irrespective of reducing agents such as sterically bulky (L-selectride or DIBAL-H at -78 ºC) and a simple and smaller ($NaBH_4$ at 0 ºC) reducing agents. The stereochemistry was unambiguously determined by the NOE experiments between the H_1 and the

H_4 protons (Scheme 4).

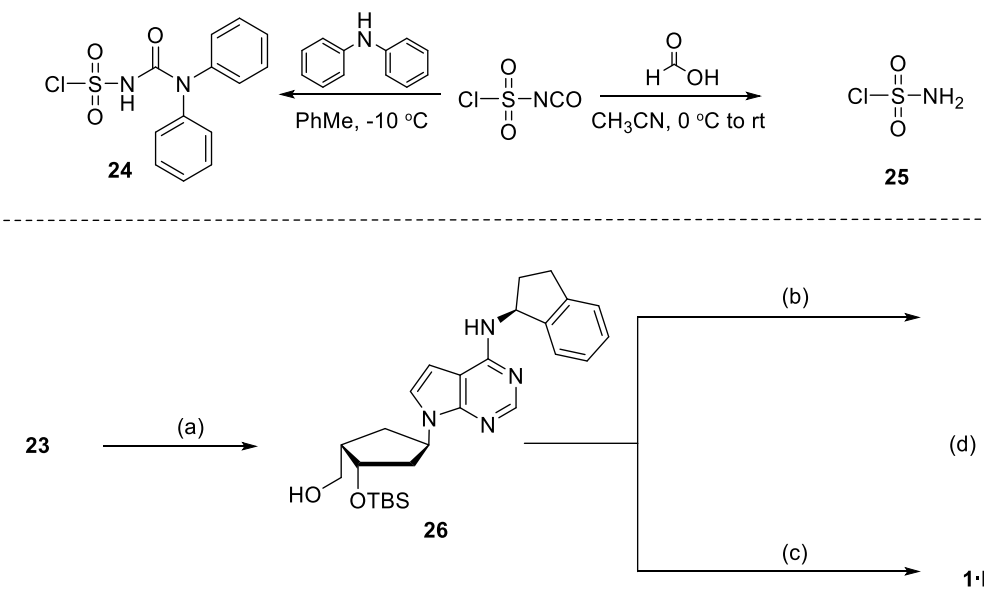


Scheme 5. Exploration of Condensation Pathways to **23.** ***Reagents and conditions*****:** (a) DIPEA, *n*-BuOH, 120 ºC, 48 h, 70%; (b) MsCl, Et_3N, CH_2Cl_2, 0 ºC, 10 min; (c) **20**, Cs_2CO_3, DMF, 90 ºC, 12 h, 75% for 2 steps; (d) **20**, PPh_3, DIAD, THF, rt to 65 ºC, 12 h; (e) **18**, PPh_3, DIAD, THF, 0 ºC to rt, 12 h, 89%; (f) **19**, DIPEA, *n*-BuOH, 120 ºC, 48 h, 61% (brsm)

With the glycosyl donor **17** in hand, we then explored the condensation pathways (Scheme 5). The nucleobase **20** was obtained by treating 7-deaza-6-chloropurine **18** with aminoindane **19** in *n*-butanol.[11b] Initial attempt was to employ Mitsunobu reaction to directly condense the nucleobase **20** with glycosyl donor **17**; however, the reaction failed to give the condensed product **23**. Surprisingly, the same Mitsunobu condensation of **17** with nucleobase **18** produced the condensed product **22** successfully, yet the substitution of **22** with aminoindane **19** resulted in low yield of **23**. Microwave irradiation also resulted in significant drop in yield of **23** due to the decomposition of **17** in such harsh condition. However, the typical S_N2

reaction[21] proved to be the most effective method for condensation. Compound **17** was converted to the mesylate **21**, which without purification was condensed with nucleobase **20** to afford the desired compound **23** in 75% yield in 2 steps.

The final step in the synthesis of **1** was to introduce the sulfamoyl group at the 5'-position (Scheme 6). Diethylaluminum chloride[22] at 0 °C cleanly removed the trityl group of **23** without affecting any other functional groups to afford alcohol **26**. The sulfamoylation of **23** with sulfamoyl chloride **25**, freshly prepared by reacting chlorosulfonyl isocyanate and formic acid,[23] afforded the desired final **1** as HCl salt, but the reaction yield was inconsistent, depending on the quantity of sulfamoyl chloride formed *in situ*.



Scheme 6. Synthesis of MLN4924 (**1**). Reagents and conditions: (a) Et_2AlCl, CH_2Cl_2, 0 °C, 30 min, 84%; (b) i) **24**, Et_3N, THF, ii) HCl, then $NaHCO_3$, THF, 0 °C to rt, 15 h, 63%; (c) **25**, Et_3N, CH_3CN, rt, 16 h, 61%; (d) HCl, EtOH, rt, 20 min, 90%

Thus, a two-step sequence was employed, using a more stable and quantifiable sulfamoylating agent **24**,[6] which was conveniently obtained by filtering the precipitate formed by reacting chlorosulfonyl isocyante and

diphenylamine in toluene.[24] Treatment of **26** with **24**, followed by deprotection with HCl afforded **1** as a free form after neutralization with sodium bicarbonate. Compound **1** was also converted to its HCl salt form upon stirring in ethanolic HCl for 20 minutes. The latter approach proved to be much more reliable for reproducibility. The spectral data of **1** as both free form and HCl salt were identical with those previously reported.[6]

3. Conclusions

In summary, an efficient and straightforward synthetic route that can be well utilized for generating structural analogues of MLN4924 was achieved via a versatile cyclopentanone intermediate through regioselective α-alkoxy removal of the isopropylidene group and stereoselective reduction as key steps. Conformational dynamics has been explored to give an insight in understanding stereoselectivity in β-substituted cyclopentanones which may account for stereoselective control in future uses. The current approach successfully afforded the MLN4924 in an overall yield of 13% in 15 steps from D-ribose which showed reliable and reproducible results.

4. Experimental Section

General Methods

Proton (^{1}H), carbon (^{13}C) NMR, spectra were recorded on a Bruker AV 400 (400/100 MHz), Bruker AMX 500 (500/125 MHz), or Jeol JNM-ECA600 (600/150 MHz). Chemical shifts are given in parts per million (ppm) (δ) relative to the solvent peak. Coupling constants (J) are reported as Hertz (Hz). High resolution mass spectra (HRMS) measurements were performed on a Thermo LCQ XP instrument. Optical rotations were determined on Jasco III in appropriate solvent. UV spectra were obtained on U-3000 made by Hitachi in methanol or water. Melting points were determined on a Buchan B-540 instrument

and are uncorrected. Reactions were checked by thin layer chromatography (Kieselgel 60 F254, Merck). Spots were visualized under UV light (254 nm), or by colorizing and charring with a *p*-anisaldehyde solution or a phosphomolybdic acid solution. The crude compounds were purified by silica gel column chromatography (Kieselgel 60, 70-230 mesh, Merck). All the anhydrous solvents were redistilled over CaH_2, P_2O_5, or sodium/benzophenone before the reaction. All reactions were performed under nitrogen atmosphere and anhydrous solvents unless specified otherwise.

2-((4*R*,5*R*)-2,2-Dimethyl-5-vinyl-1,3-dioxolan-4-yl)-1-(trityloxy)but-3-en-2-ol (11)[16]

Compound **11** was obtained from D-ribose in 72% overall yield over 5 steps. An additional column purification can be done after Swern oxidation to reduce the amount of Grignard reagent required for subsequent step. All spectra were in accordance with the previously reported literature.[16]

(3a*R*,6a*R*)-2,2-Dimethyl-6-((trityloxy)methyl)-3a,6a-dihydro-4*H*-cyclopenta[*d*][1,3]dioxol-4-one (13)[16]

To a stirred solution of **11** (22 g, 48.3 mmol) in dry toluene (1 L) was added Neolyst M2 (0.89 g, 0.97 mmol) under nitrogen atmosphere and the resulting reaction mixture was stirred at room temperature for 2 days. The residue was filtered through a pad of silica and the volatiles were removed *in vacuo*. The crude product **12** was used without any further purification.

To a stirred solution of **12** (11 g, 427.24 mmol) described above in anhydrous dichloromethane (1 L) was added pyridinium dichromate (48.4 g, 129 mmol) and 4Å molecular sieves (10 g) and the resulting reaction mixture was vigorously stirred at room temperature for 18 hours. The mixture was filtered through a mixed pad of Celite and silica, and the filter

cake was thoroughly washed with dichloromethane (200 mL x 3). The crude residue was purified by silica gel column chromatography eluting hexane/ethyl acetate (4:1) to give **13** as a white solid (13 g, 63% for 2 steps). All spectra were in accordance with the previously reported literature.[16]

(3a*R*,6*S*,6a*R*)-2,2-Dimethyl-6-((trityloxy)methyl)tetrahydro-4*H*-cyclopenta[*d*][1,3]dioxol-4-one (14)[17]

To a solution of **13** (41 g, 96 mmol) in ethyl acetate (480 mL) was added 10% palladium on activated carbon (1.02 g, 9.6 mol), followed sodium carbonate (41 g, 384mmol) and the resulting suspension was stirred under hydrogen atmosphere at room temperature overnight. The mixture was filtered through a pad of Celite, thoroughly washed with ethyl acetate twice (250 mL x 2) and the filtrate was concentrated under reduced pressure to give **14** (40.3 g, 98%) as a colorless solid. All spectra were in accordance with the previously reported literature.[15] $[\alpha]_D^{20}$ = -81.4 (*c* 0.1, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 7.49-7.42 (m, 6H), 7.33-7.26 (m, 6H), 7.25-7.20 (m, 3H), 4.86 (merged dd, $J_1 = J_2$ = 4.2 Hz, 1H), 4.21 (d, J = 4.7 Hz, 1H), 3.50 (merged dd, $J_1 = J_2$ = 8.6 Hz, 1H), 3.21 (dd, J_1= 8.6 Hz, J_2 = 6.7 Hz, 1H), 2.49-2.40 (m, 1H), 2.29 (J_{AB} = 18.4 Hz, J_2 = 7.7 Hz, 1H), 2.17 (J_{AB} = 18.4 Hz, J_2 = 12.5 Hz, 1H), 1.35 (s, 3H), 1.33(s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 213.9, 143.9, 128.6, 127.7, 126.9, 112.3, 86.6, 80.1, 77.5, 62.9, 37.0, 35.9, 26.8, 25.1; HRMS (ESI): found 429.2070 [calcd for $C_{18}H_{29}O_4^+$ $(M + H)^+$ 429.2062].

(3*S*,4*S*)-3-Hydroxy-4-((trityloxy)methyl)cyclopentan-1-one (15)

Preparation of aluminum amalgam: The preparation of aluminum amalgam is an exothermic reaction with evolution of gas involved and thus the reaction should be handled with extreme care. The reaction was conducted

in several batches because larger scale reactions tend to cause stirring issues due to the aggregation of excess solid aluminum amalgam present.

To a stirred solution of saturated aqueous mercury(II) chloride (30 mL) was added granular aluminum (5 g) and the resulting gray suspension was stirred at room temperature for 5 minutes. The reaction mixture was filtered through a pad of Celite, washed successively with distilled water, ethanol and ether. The solid aluminum amalgam was used immediately without any further purification.

To a stirred solution of **14** (10.1 g, 23.6 mmol) in THF/H_2O (180 mL, 160 mL THF, 20 mL H_2O, 8:1 v/v) was added freshly prepared aluminum amalgam described above in several portions and the resulting reaction mixture was vigorously stirred at room temperature. After 3 hours, additional portion of aluminum amalgam, freshly prepared, was added and the reaction mixture was left to stir for additional 3 hours at room temperature. The gray viscous mixture was filtered through a pad of Celite and the filtrate was concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography eluting hexane/ethyl acetate (3:1) to afford **15** (6.99 g, 80%) as a white solid.

$[\alpha]_D^{20}$ = -30.5 (*c* 0.79, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.43 (d, J = 7.3 Hz, 6H), 7.31 (t, J = 7.3 Hz, 6H), 7.26-7.23 (m, 3H), 4.68 (s, 1H), 3.52 (merged dd, J_1 = J_2 = 4.8 Hz, 1H), 3.24 (merged t, J_1= 8.4 Hz, J_2 = 9.5 Hz, 1H), 2.51 (m, 1H), 2.37-2.32 (m, 2H), 2.18 (d, J = 10.0 Hz, 2H), 2.10 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 216.3, 143.5, 128.4, 128.0, 127.3, 87.0, 70.3, 62.4, 47.6, 41.9, 37.8; HRMS (FAB+): found 372.1707 [calcd for $C_{25}H_{24}O_3^+$ $(M + H)^+$ 372.1725].

(3*S*,4*S*)-3-((*tert*-Butyldimethylsilyl)oxy)-4-((trityloxy)methyl)cyclop-entan-1-one (16)

To a stirred solution of **15** (12.4 g, 33.3 mmol) and imidazole (9.07 g, 133 mmol) in dry DMF (150 mL) was added *tert*-butyldimethylsilyl chloride (10

g, 66.6 mmol) at 0 ºC and the resulting reaction mixture was stirred at room temperature under nitrogen atmosphere for 24 hours. The reaction mixture was diluted with water (300 mL) and the aqueous phase was extracted with diethyl ether (150 mL x 3). The combined organic layers were washed with brine, dried over anhydrous magnesium sulfate, filtered and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography eluting hexane/ethyl acetate (5:1) to afford **16** (14.3 g, 88%) as a colorless oil. $[\alpha]_D^{20}$ = 28.6 (*c* 0.539, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.41 (d, *J* = 7.4 Hz, 6H), 7.28 (t, *J* = 7.2 Hz, 6H), 7.24-7.21 (m, 3H), 4.51 (s, 1H), 3.27 (m, 2H), 2.47 (m, 1H), 2.37-2.32 (m, 2H), 2.27-2.23 (m, 1H), 2.17-2.12 (m, 1H), 0.68 (s, 9H), -0.05 (s, 3H), -0.14 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 216.8, 144.1, 128.7, 127.7, 127.0, 86.8, 70.4, 63.8, 49.0, 43.6, 39.4, 25.6, 17.8, -4.7, -5.2; HRMS (FAB+): found 486.2573 [calcd for $C_{31}H_{38}O_3Si^+$ $(M + H)^+$ 486.2590].

(1*S*,3*S*,4*S*)-3-((*tert*-Butyldimethylsilyl)oxy)-4-((trityloxy)methyl)cyclopentan-1-ol (17)

***Method A (L-selectride)*:** To a stirred solution of **16** (293 mg, 0.60 mmol) in anhydrous THF (6 mL) was dropwise added L-selectride 1.0 M in THF solution (0.78 mL, 0.78 mmol) at -78 ºC under nitrogen atmosphere and the resulting solution was stirred at same temperature for 10 minutes. The reaction was quenched with saturated aqueous ammonium chloride, diluted with ethyl acetate (10 mL) and poured into water (15 mL). The layers were separated, and the aqueous layer was extracted with ethyl acetate twice (10 mL x 2). The combined organic layers were washed with brine, dried over anhydrous magnesium sulfate, filtered and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography eluting hexane/ethyl acetate (3:1) to afford **17** (260 mg, 89%) as a colorless oil.

Method B (sodium borohydride): To a stirred solution of **16** (62 mg, 0.13 mmol) in methanol (2 mL) was added sodium borohydride (6.4 mg, 0.17

mmol) at 0 °C and the reaction mixture was stirred at same temperature for 10 minutes. The reaction was quenched with water, diluted with ethyl acetate (5 mL) and diluted with water (10 mL). The aqueous layer was extracted with ethyl acetate (10 mL x 2) and the combined organic layers were washed with brine, dried over anhydrous magnesium sulfate, filtered and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography eluting hexane/ethyl acetate (3:1) to afford **17** (58 mg, 91%) as a colorless oil. $[\alpha]_D^{20}$ = 20.6 (*c* 0.39, MeOH);^{1}H NMR (500 MHz, $CDCl_3$): δ 7.41 (d, *J* = 7.5 Hz, 6H), 7.27 (t, *J* = 7.2 Hz, 6H), 7.24-7.21 (m, 3H), 4.29 (s, 1H), 4.23 (bs, 1H), 3.23-3.16 (m, 2H), 2.64 (bs, 1H), 2.43-2.36 (m, 1H), 2.12-2.08 (m, 1H), 1.85-1.82 (m, 1H), 1.79-1.74 (m, 1H), 1.49 (m, 1H), 0.70 (s, 9H), -0.05 (s, 3H), -0.17 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 144.3, 128.7, 127.7, 126.9, 86.6, 74.9, 73.2, 64.5, 46.0, 44.8, 38.7, 25.7, 17.8, -4.8, -5.3; HRMS (ESI): found 511.2638 [calcd for $C_{31}H_{40}NaO_3Si^+$ $(M + Na)^+$ 511.2639].

7-((1*R*,3*S*,4*S*)-3-((*tert*-Butyldimethylsilyl)oxy)-4-((trityloxy)methy-l)cyclopentyl)-*N*-((*S*)-2,3-dihydro-1H-inden-1-yl)-7*H*-pyrrolo[2,3-d]-pyrimidin-4-amine (23)

To a solution of **17** (207 mg, 0.42 mmol) in dry dichloromethane (5 mL) was added triethylamine (0.09 mL, 0.63 mmol) followed my methanesulfonyl chloride (0.04 mL, 0.50 mmol) dropwise at 0 °C under nitrogen atmosphere and the resulting solution was stirred at 0 °C for 10 minutes. The reaction was poured into water (10 mL) and the aqueous layer was extracted with dichloromethane (5 mL x 2). The combined organic layers were washed with brine, dried over anhydrous magnesium sulfate, filtered and concentrated *in vacuo*. The crude mesylate **21** obtained was used immediately without any further purification.

To a solution of **20** (138 mg, 0.55 mmol) in dry DMF (5 mL) was added cesium carbonate (411 mg, 1.26 mmol) under nitrogen atmosphere and the

resulting reaction mixture was stirred at room temperature for 1 hour. The solution was cooled to 0 ºC and freshly prepared mesylate **21** described above was dropwise added. Upon completion of addition, the reaction mixture was heated to 90 ºC and stirred overnight. The solution was cooled to room temperature and was diluted with water (20 mL), and the aqueous phase was extracted with diethyl ether (10 mL x 3). The combined organic layers were washed with brine, dried over anhydrous magnesium sulfate, filtered and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography eluting hexane/ethyl acetate (2:1) to give **23** as a colorless oil. (228 mg, 75% in 2 steps). $[\alpha]_D^{20}$ = 28.6 (*c* 0.53); UV (CH_3OH) λ_{max} 273.6 nm; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.40 (s, 1H), 7.42 (d, *J* = 7.4 Hz, 6H), 7.36 (d, *J* = 7.4 Hz, 1H), 7.28-7.20 (m, 12H), 6.98 (d, *J* = 3.6 Hz, 1H), 6.34 (s, 1H), 5.90 (m, 1H), 5.42 (m, 1H), 5.27 (bs, 1H), 4.42 (s, 1H), 3.28-3.25 (m, 1H), 3.16 (t, *J* = 8.2 Hz, 1H), 3.05-3.01 (m, 1H), 2.97-2.92 (m, 1H), 2.74 (m, 1H), 2.57 (m, 1H), 2.29-2.24 (m, 2H), 2.15-2.03 (m, 2H), 2.00-1.96 (m, 1H), 0.73 (s, 9H), -0.03 (s, 3H), -0.16 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 156.0 151.6 149.9 144.3, 143.8, 143.6, 128.7, 128.0, 127.7, 126.9, 126.8, 124.9, 124.3, 121.5, 103.0, 97.7, 86.6, 73.8, 64.1, 56.2, 52.8, 45.7, 43.3, 34.8, 34.7, 30.2, 25.7, 17.9, -4.6, -5.2; HRMS (FAB+): found 721.3947 [calcd for $C_{46}H_{53}N_4O_2Si^+$ $(M + H)^+$ 721.3938].

((1*S*,2*S*,4*R*)-2-((*tert*-Butyldimethylsilyl)oxy)-4-(4-(((*R*)-2,3-dihydro-1-*H*-inden-1-yl)amino)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)cyclopent-yl)methanol (26)

To a stirred solution of **23** (121 mg, 0.17 mmol) in dry dichloromethane (3 mL) was dropwise added diethylaluminum chloride (0.90 M in toluene solution, 0.68 mL, 0.68 mmol) at 0 ºC and the resulting reaction mixture was stirred for 30 minutes. The reaction was quenched with saturated aqueous ammonium chloride (3 mL) at 0 ºC and was vigorously stirred for 30 minutes at room temperature. The layers were separated, and the

aqueous layer was extracted with dichloromethane twice (3 mL x 2). The combined organic layers were washed with brine, dried over anhydrous magnesium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography eluting hexane/ethyl acetate (2:1) to afford **26** (68 mg, 84%) as a colorless oil. $[\alpha]_D^{20}$ = -7.45 (*c* 1.02); UV (CH_3OH) λ_{max} 280 nm; ^1H NMR (500 MHz, $CDCl_3$): δ 8.37 (s, 1H), 7.34 (d, *J* = 7.4 Hz, 1H), 7.27-7.23 (m, 1H), 7.20-7.17 (t, *J* = 7.3 Hz, 1H), 6.92 (d, *J* = 3.6 Hz, 1H), 6.29 (d, *J* = 2.7 Hz, 1H), 5.87 (m, 1H), 5.35 (m, 1H), 5.21 (bs, 1H), 4.62 (q, , *J* = 4.4 Hz, 1H), 3.79-3.74 (m, 2H), 3.05-3.00 (m, 1H), 2.95-2.88 (m, 1H), 2.75-2.69 (m, 1H), 2.56-2.51 (m, 1H), 2.40-2.33 (m, 1H), 2.27 (q, *J* = 4.0 Hz, 2H), 2.04-1.93 (m, 2H), 0.91 (s, 9H), 0.11 (d, *J* = 3.1Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 155.9 151.5 149.8 143.7, 143.5, 128.0, 126.7, 124.8, 124.2, 121.6, 103.2, 97.6, 75.2, 62.6, 56.1, 53.4, 45.5, 42.6, 34.6, 33.0, 30.2, 25.8, 18.0, -4.5, -5.1; HRMS (FAB+): found 479.2846 [calcd for $C_{27}H_{39}N_4O_2Si]^+$ $(M + H)^+$ 479.2842].

((1*S*,2*S*,4*R*)-4-(4-(((*S*)-2,3-dihydro-1*H*-inden-1-yl)amino)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)-2-hydroxycyclopentyl)methyl sulfamate (1)[6]

To a stirred solution of **26** (82 mg, 0.17 mmol) in dry tetrahydrofuran (5 mL) was added triethylamine (0.03 mL, 0.22 mmol) followed by **24** (69 mg, 0.22 mmol) at 0 ºC and the reaction mixture was stirred at room temperature under nitrogen atmosphere for 2 hours. 2.0 M HCl(aq.) was (0.43 mL, 0.86 mmol) added to the solution and the resulting solution was stirred at room temperature overnight. The reaction was quenched and neutralized with saturated aqueous sodium bicarbonate solution and was vigorously stirred for 1 hour. The solution was extracted with ethyl acetate thrice (15 mL x 3) and the combined organic layers were washed with brine, dried over anhydrous magnesium sulfate, filtered, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography eluting dichlormethane/methanol (95:5) to afford **1** as a coloress solid (48 mg, 63%).

All spectra were in accordance with the previously reported literature.[6] $[\alpha]_D^{20}$ = (*c* 0.37); UV (CH_3OH) λ_{max} 273.3; ^1H NMR (500 MHz, CD_3OD): δ 8.16 (s, 1H), 7.26-7.24 (m, 2H), 7.21-7.13 (m, 3H), 6.62 (d, *J* = 3.6 Hz, 1H), 5.85 (t, *J* = 7.6 Hz, 1H), 5.45 (m, 1H), 4.49 (m, 1H), 4.37 (td, J_1 = 2.0 Hz, J_2 = 7.7 Hz, 1H), 4.19 (td, J_1 = 2.1 Hz, J_2 = 7.5 Hz, 1H), 3.08-3.02 (m, 1H), 2.94-2.88 (m, 1H), 2.79 (m, 1H), 2.66-2.60 (m, 1H), 2.35-2.30 (m, 1H), 2.27-2.20 (m, 2H), 2.06-1.97 (m, 2H); ^{13}C NMR (125 MHz, CD_3OD): δ 158.6, 152.8, 150.8, 146.0, 145.4, 129.5, 128.3, 126.5, 126.0, 123.3, 105.6, 101.2, 73.8, 71.6, 57.7, 54.7, 45.5, 44.3, 35.7, 35.3, 31.8; HRMS (FAB+): found 444.1712 [calcd for C21H26N5O4S]+ (M + H)+ 444.1706].

((1*S*,2*S*,4*R*)-4-(4-(((*S*)-2,3-dihydro-1*H*-inden-1-yl)amino)-7*H*-pyrrolo[2,3-d]pyrimidin-7-yl)-2-hydroxycyclopentyl)methyl sulfamate, HCl Salt (1·HCl) [6]

To a stirred solution of **1** (32 mg, 0.07 mmol) in ethanol (3 mL) was added HCl 1.25 M ethanol solution (0.3 mL, 0.04 mmol) and the resulting solution was stirred at room temperature for 30 minutes. The volatiles were removed *in vacuo* to afford **1·HCl** (31 mg, 90%) as a colorless solid, of which the ^1H-NMR spectra were in accordance with the previously reported literature.[6]

5. References

1. Rethinking the war on cancer, https://www.newsweek.com/rethinking-war-cancer-88941 (accessed April 2019);
2. Advances Elusive in the Drive to Cure Cancer, https://www.nytimes.com/2009/04/24/health/policy/24cancer.html (accessed April 2019)
3. (a) A. Mullard, *Nat Rev Drug Discov.* **2019**, *18*, 85; (b) Novel Drug Approved for 2018, https://www.fda.gov/drugs/developmentapprovalprocess/druginnovation

/ucm592464.htm (accessed April 2019); (c) Drugs Approved in 2018, https://cen.acs.org/sections/drugs-approved-in-2018/index.html (accessed April 2019)

4. V. Schirrmacher, *Int. J. Onc.*, **2019**, *54*, 407.
5. (a) B. Vogelstein, N. Papadopoulos, V. E. Velculescu, S. Zhou, L. A. Diaz Jr., and K. W. Kinzler, *Science*, **2013**, *339*, 1546.; (b) M. P. Patricelli, M. R. Janes, L-S. Li, R. Hansen, U. Peters, L. V. Kessler, Y. Chen, J. M. Kucharski, J. Feng, T. Ely, J. H. Chen, S. J. Firdaus, A. Babbar, P. Ren and Y. Liu, *Cancer Discov.*, **2016**, *6*, 316.; (c) W. C. Gustafson, J. G. Meyerowitz, E. A. Nekritz, J. Chen, C. Benes, E. Charron, E. F. Simonds, R. Seeger, K. K. Matthay, N. T. Hertz, M. Eilers, K. M. Shokat and W. A. Weiss, *Cancer Cell*, **2014**, *26*, 414.; (d) L. N. Makley and J. E. Gestwicki, *Chem. Biol. Drug Des*. **2013**, *81*, 22.; (e) S. P. Velagapudi, M. G. Costales, B. R. Vummidi, Y. Nakai, A. J. Angelbello, T. Tran, H. S. Haniff, Y. Matsumoto, Z. F. Wang, A. K. Chatterjee, J. L. Childs-Disney, and M. D. Disney, *Cell Chem. Biol.*, **2018**, *25*, 1086.; (f) C. V. Dang, E. P. Reddy, K. M. Shokat and L. Soucek, *Nat. Rev. Cancer*, **2017**, *17*, 502.
6. T. A. Soucy, P. G. Smith, M. A. Milhollen, A. J. Berger, J. M. Gavin, S. Adhikari, J. E. Brownell, K. E. Burke, D. P. Cardin, S. Critchley, C. A. Cullis, A. Doucette, J. J. Garnsey, J. L. Gaulin, R. E. Gershman, A. R. Lublinsky, A. McDonald, H. Mizutani, U. Narayanan, E. J. Olhava, S. Peluso, M. Rezaei, M. D. Sintchak, T. Talreja, M. P. Thomas, T. Traore, S. Vyskocil, G. S. Weatherhead, J. Yu, J. Zhang, L. R. Dick, C. F. Claiborne, M. Rolfe, J. B. Bolen and S. P. Langston, *Nature*, **2009**, *458*, 732.
7. G. Xu, J. I. Toth, S. R. da Silva, S. L. Paiva, J. L. Lukkarila, R. Hurren, N. Maclean, M. A. Sukhai, R. N. Bhattacharjee, C. A. Goard, B. Medeiros, P. T. Gunning, S. Dhe-Paganon, M. D. Petroski and A. D. Schimmer, *PloS ONE* **2014**, *9*, e93530

8. F. S. Wolenski, C. D. Fisher, T. Sano, S. D. Wyllie, L. A. Cicia, M. J. Gallacher, R. A. Baker, P. J. Kirby and J. J. Senn, *Cell Death Discov.*, **2015**, *1*, 15034.
9. R. Swords, H. P. Erba, D. J. DeAngleo, D. L. Bixby, J. K. Altman, M. Maris, Z. Hua, S. J. Blakemore, H. Faessel, F. Sedarati, B. J. Dezube, F. J. Giles and B. C. Medeiros, *Br. J. Haematol.*, **2015**, *169*, 534.
10. (a) L. Li, B. Liu, T. Dong, H.W. Lee, J. Yu, Y. Zheng, H. Gao, Y. Zheng, H. Gao, Y. Zhang, Y. Chu, G. Liu, W. Niu, S. Zheng, L. S. Jeong and L. Jia, *Biochem. Biophys. Res. Commun.,* **2013**, *432***,** 494.; (b) W. T. Yao, J. F. Yu, G. Y. Yu, R. Wang, K. Wang, L. H. Li, P. Chen, Y N. Jiang, H. Cheng, H. W. Lee, J. Yu, H. Qi, X. J. Yu, P. Wang, Y. W. Chu, M. Yang, Z. C. Hua, H. Q. Ying, R. M. Hoffman, L. S. Jeong, L. J. Jia, *Cell Death Dis.*, **2014**, *5*, e1509.; (c) P. Chen, T. Hu, Y. Liang, P. Li, X. Chen, J. Zhang, Y. Ma, Q. Hao, J. Wang, P. Zhang, Y. Zhang, H. Zhao, S. Yang, J. Yu, L. S. Jeong, H. Qi, M. Yang, R. M. Hoffman, Z. Dong and L. Jia, *Clin. Cancer Res.*, **2016**, *22*, 4145.; (d) X. Wang, W. Zhang, Z. Yan, Y. Liang, L. Li, X. Yu, Y. Feng, S. Fu, Y. Zhang, S. Fu, Y. Zhang, H. Zhao, J. Yu, L. S. Jeong, X. Guo and L. Jia, *Oncotarget*, **2016**, *7*, 38380.; (e) J. Wang, S. Wang, W. Zhang, X. Wang, X. Liu, L. Liu, L. Li, Y. Liang, J. Yu, L. S. Jeong, L. Jia, H. Zhao and Y. Zhang, *Biochem. Biophys. Res. Commun.*, **2017**, *490*, 1183.; (f) W. Zhang, Y. Liang, L. Li, X. Wang, Z. Yan, C. Dong, M. S. Zeng, Q. Zhong, X. K. Liu, J. Yu, S. Sun, X. Liu, J. Kang, H. Zhao, L. S. Jeong, Y. Zhang and L. Jia, *Cell Prolif.*, **2019**, *52*, e12536.
11. (a) S. Critchley, T. G. Gant, S. P. Langston, E. J. Olhava and S. Peluso, PCT Int. Appl. WO 2006084281 Al, 2006.; (b) S. P. Langston, E. J. Olhava and S. Vyskocil, PCT Int. Appl. WO 2007092213 A2, 2007.
12. H. W. Lee, S. K. Nam, W. J. Choi, H. O. Kim and L. S. Jeong, *J. Org. Chem.*, **2011**, *76*, 3557.
13. I. Armitage, E. L, Elliott, M. Langston, S. P. Langston, Q. J. McCubbin,

H. Mizutani, M. Sitrling and L. Zhu, U.S. Pat. Appl. Publ. 20090036678, 2009.

14. I. Armitage, E. L. Elliott, F. Hicks, M. Langston, A. McCarron, Q. J. McCubbin, E. O'Brien, M. Stirling and L. Zhu, *Org. Process Res. Dev.*, **2015**, *19*, 1299.
15. M. J. Robins, J. S. Wilson and F. Hansske, *J. Am. Chem. Soc.*, **1983**, *105*, 4059.
16. W. J. Choi, H. R. Moon, H. O. Kim, B. N. Yoo, J. A. Lee, D. H. Shin and L. S. Jeong, *J. Org. Chem.*, **2004**, *69*, 2634.
17. D. B. Jarhad, M. H. Jang, Y. S. Shin, G. Kim, H.-R. Kim, Y. E. Hyun, J.-S. Yoon and L. S. Jeong, *Org. Chem. Front.*, **2019**, *6*, 959-966.
18. J. Inanaga, J. Katsuki and M. Yamaguchi, *Chem Lett*, 1991, **20**, 1025.
19. G. A. Molander and G. Hahn, *J. Org. Chem.*, **1986**, *51*, 1135.
20. C. R. Johnson and T. D. Penning, *J. Am. Chem. Soc.*, **1988**, *110*, 4726.
21. Y. Li, M. Plesescu and S. R. Prakash, *Tetrahedron Lett.*, **2011**, *52*, 1807.
22. H. Köster and N. D. Sinha, *Tetrahedron Lett.*, **1982**, *23*, 2641.
23. R. Appel and G. Berger, *Chem. Ber.* **1958**, *91*, 1339.
24. M. Ghiasi and S. Kamalinahad, *J. Carbohydr. Chem.*, **2015**, *34*, 80.

Part IV.

Asymmetric Synthesis of Fluoro-MLN4924 as a Selective NEDD8-Activating Enzyme (NAE) Inhibitor

1. Introduction

Protein homeostasis plays a crucial role in maintaining proper cellular functions within the human body and failure to do so affects diverse physiological pathways that may eventually result in the progression to cancer.[1,2] Eukaryotic cells are known to preserve cellular homeostasis by ubiquitin-proteasome system (UPS), of which the process is regulated by proteasome-mediated degradation of unwanted, polyubiquitin-tagged proteins.[3] The role of UPS in tumor growth and survival have been discovered[4] and the fact that proteasome inhibitor bortezomib reaching the market as a treatment of multiple myeloma and relapsed mantle cell lymphoma implies ubiquitin-proteasome system is a valid target for anticancer therapy;[5] however, because proteasome inhibition is known to interfere in multiple cellular pathways,[6] exploring UPS for therapeutic use with high target selectively yet remains an uncompleted task.

Ubiquitination has also been an attractive topic for investigation in the past years[7] and during the course, ubiquitin-like proteins (Ubls) such as NEDD8 has been identified as a fresh target for therapeutic uses.[8] The inhibition of NEDD8 is known to inactivate cullin-RING ligases (CRLs) which affect diverse cellular pathways including DNA replication/repair, nuclear factor signaling, and hypoxia signaling.[9] Due to the importance of CRLs in tumor growth,[9] inhibition of the NEDD8-activating enzyme (NAE) has been proposed as a promising target for anticancer therapeutics.[10]

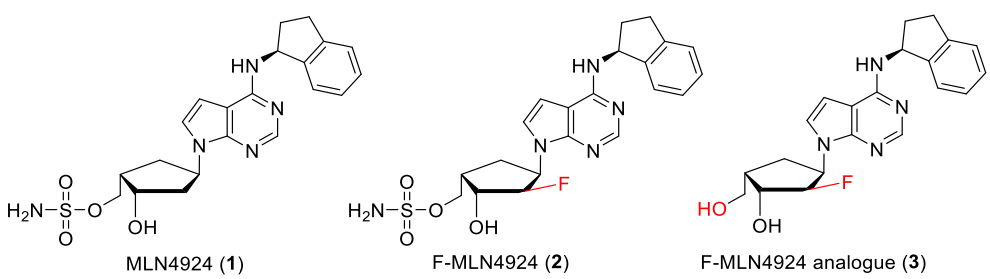
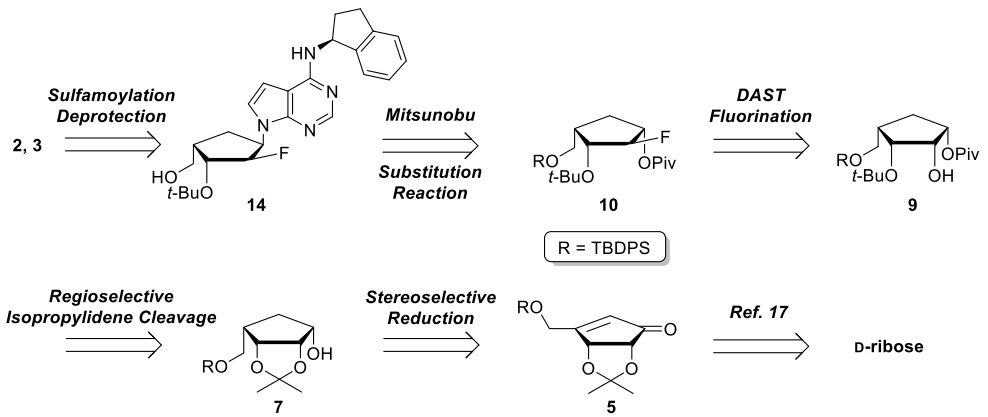


Figure 1. Structures of MLN4924 (**1**), F-MLN4924 (**2**) and its analogue (**3**)

MLN4924 (**1**), also known as pevonedistat, is a first-in-class small molecule that selectively inhibits NAE, which is currently undergoing clinical trials for the use as an anticancer agent.[11] MLN4924 binds to NEDD8 by acting as an adenosine monophosphate (AMP) mimetic, which forms a stable NEDD8-MLN4924 adduct that prevents the activation of NAE which in turn inactivates cullin-RING ligases, eventually leading to apoptosis in cancer cells.[12] Due to its potent anticancer activity with unique structural aspects including an unnatural 4-L-configuration with chiral indane moeity, **1** is expected to serve as a previliged template for diverse biological uses.

In continuation to our efforts in conducting a comprehensive structure-activity relationship studies of **1**, we were interested in incorporating fluorine as a bioisostere of hydrogen, which has proven to be an effective strategy in modern drug discovery,[13,14] since the introduction of fluorine often enhances binding affinity and metabolic stability due to its different physicochemical nature[15] with respect to hydrogen. To this end, we were interested in preparing fluroinated MLN4924 analouges, with fluorine at the C2 in an *arabino* configuration in particular, based on the success of clofarabine development from cladribine;[16] thus we have designed fluorinated analogue **2** accordingly. Furthermore, we have also designed compound **3** to see whether conversion of these fluorinated analogue to its corresponding triphosphate would still possess anticancer properties, by which will corroborate whether the sulfamoyl moeity is necessary for biological activity.

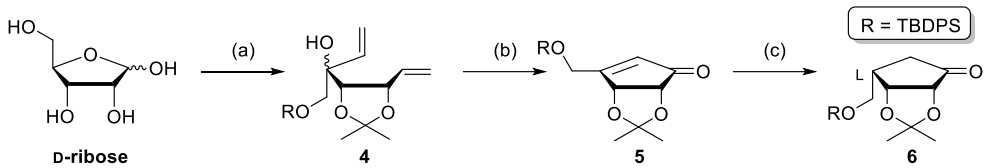
2. Results and Discussion



Scheme 1. Retrosynthetic analysis of **2** and **3**

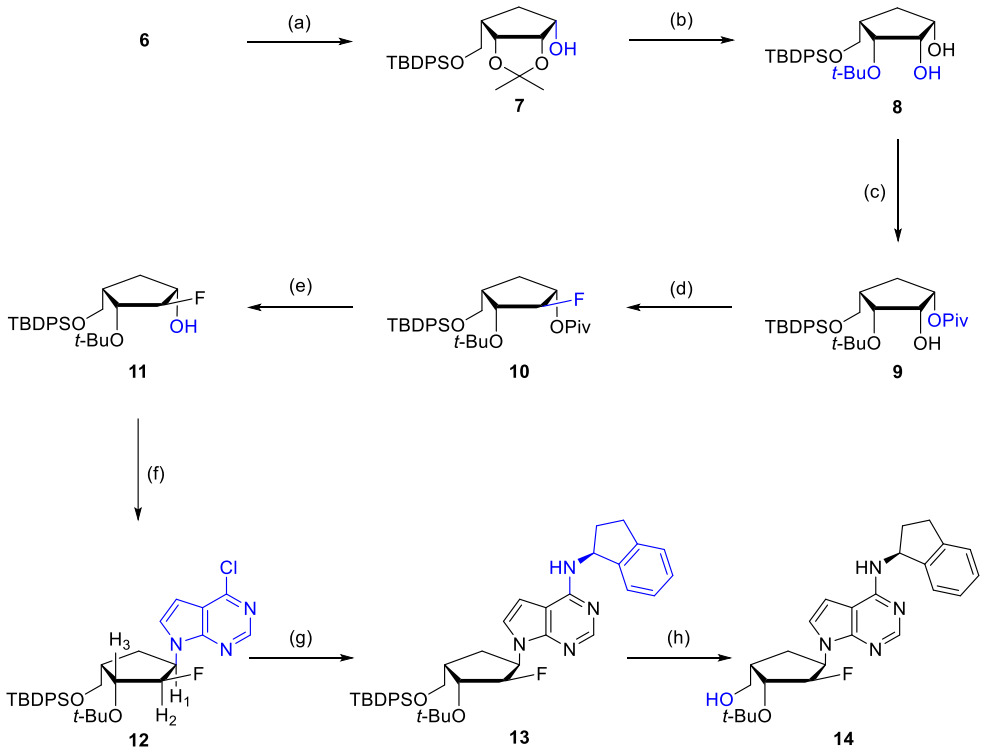
In order to provide 2'-β-fluoro MLN4924 and its analogue, a reliable synthetic route had to be secured. We envisioned that **2** and **3** can be synthesized from a common intermediate **14**, which we planned on gaining access by employing stereoselective reduction, regioselective isopropylidene cleavage and DAST fluroination as key steps from a known carbocyclic sugar intermediate **5**. (Scheme 1)

While we have previously reported an efficient conversion of D-ribose to **5**,[17] (Scheme 1) we have realized from a 25 gram-scale synthesis that the amount of reagents used in combination with the amount of silica gel and eluents required for purification processes were substantial, which needed improvement for large scale reactions for further library expansion. Our approach to this issue involved avoiding silica gel column purification whenever possible, mostly by altering work up processes and replacing costly reagents to a cheaper alternative. As a result, we have modified the previously reported protocol in a cost-efficient manner, which successfully provided intermediate **5** not only in an economical way but also in a shorter period of time.



Scheme 2. Preparation of 4-L-carbocyclic template **6**. Reagents and Conditions: (a) i) *c*-H_2SO_4, acetone, rt, 2.5 h; ii) TBDPSCl, DMAP, Et_3N, CH_2Cl_2, rt, 16 h; iii) CH_3PPh_3Br, KO*t*-Bu, THF, 0 ºC to rt, 12 h; iv) $(COCl)_2$, DMSO, CH_2Cl_2, - 78 ºC, 1 h, then Et_3N, rt, 1 h; v) vinylmagnesium bromide, THF, -78 ºC to rt, 3 h, 57%; (b) i) Neolyst M2, toluene, 60 ºC, 15 h; ii) PDC, 4Å MS, DMF, rt, 18 h, 71%; (c) H_2, Pd/C, MeOH, 16 h, 99%

D-ribose was protected with acetonide and TBDPS, which successively underwent Wittig reaction, Swern oxidation and Grignard reaction with vinylmagnesium bromide to afford crude diene **4**. The reactions all went smoothly with a simple filtration and/or extraction work ups. In order to reduce the amount of catalyst loading for ring-closing metathesis (RCM), silica gel column purification was performed at this stage to give **4** in a 5-step yield of 57% from D-ribose. **4** underwent RCM reaction with Neolyst M2, an economical alternative to Grubbs' catalyst, followed by oxidative rearrangement provided the carbocyclic sugar intermediate **5** in 71% yield in 2 steps. The 4-L-configuration was installed by hydrogenation reaction utilizing facial selectivity[18] to give **6** in a quantitative yield.



Scheme 3. Synthesis of intermediate **14**. Reagents and Conditions: (a) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, MeOH, 0 °C to rt, 30 min, 98%; (b) Me_3Al, CH_2Cl_2, 0 °C to rt, 48 h, 62%; (c) pivaloyl chloride, DIPEA, DMAP, CH_2Cl_2, rt, 16 h, 88%; (d) DAST, pyridine, CH_2Cl_2, 0 °C to rt, 3 h, 80%; (e) NaOMe, MeOH, 50 °C, 16 h, 99%; (f) 7-deaza-6-chloropurine, PPh_3, DIAD, THF, rt, 18 h, 76%; (g) (*S*)-1-aminoindane hydrochloride, DIPEA, *n*-BuOH, 90 °C, 48 h, 76%; (h) TBAF, THF, 0 °C to rt, 16 h, 97%

With **6** in hand, we then turned our attention to preparing the key intermediate **14** as shown in Scheme 3. Stereoselective reduction was achieved by employing sodium borohydride in the presence of cerium chloride to yield **7** as a single stereoisomer.[18] Trimethylaluminum-mediated regioselective isopropylidene cleavage furnished diol **8**,[18] of which the hydroxyl group at the C1 position was selectively protected with pivaloyl chloride to give **9**. The key fluorination reaction was achieved using DAST[19] as the fluorine source which smoothly produced fluorinated sugar **10**. Deprotection of the pivaloyl group with sodium methoxide furnished the glycosyl donor **11** with an excellent yield.

After securing the glycosyl donor **11**, we then focused on condensation

pathways. Our initial approach to **13** included direct condensation of the nucleobase moeity of MLN4924 with the glycosyl donor **11** under Mitsunobu condition, however the reaction failed to produce **13**. Interestingly, the condensation of 6-chloro-7-deazapurine with the glycosyl donor **11** under the same Mitsunobu protocol successfully afforded the 6-chloro-7-deaza nucleoside **12**.

While DAST reaction is known to undergo an inversion of stereochemistry,[19] it was worth conducting a 2D NOESY experiment on **12** to confirm whether the stereochemistry has been correctly assigned. The correlation between the characteristic 2' proton (indicated as H_2 in Scheme 3, assigned on the basis of geminal fluorine-proton coupling constant, $J_{H\text{-}F}$ = 53.4 Hz) and the neighboring H_1 indicated their respective close spatial relationships; however, the presence of NOE cross-peak between H_2 and H_3, despite their expected-to-be *trans*-relationship, prevented us from drawing any clear conclusion.

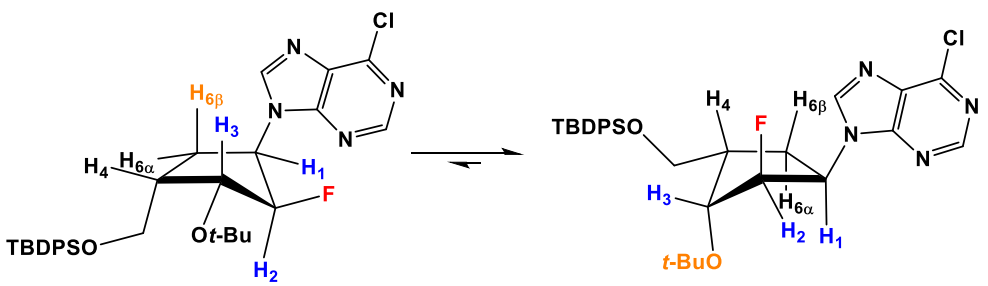
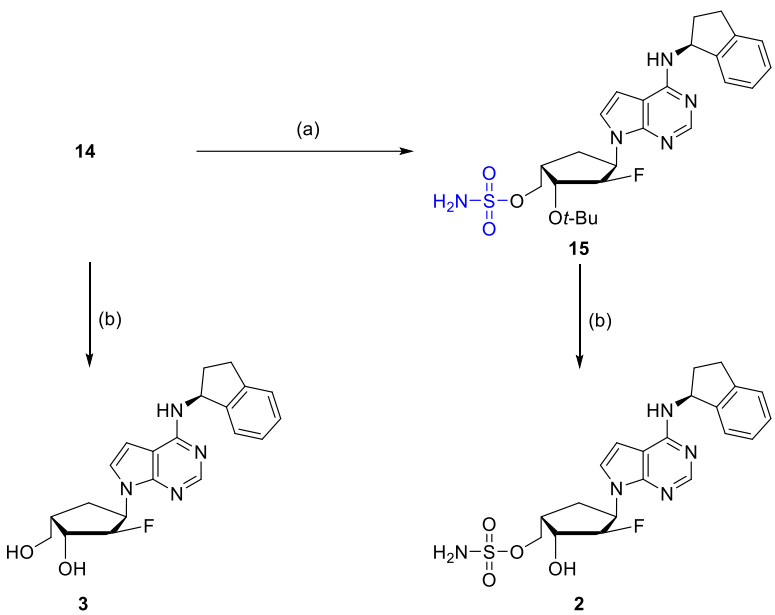


Figure 2. 2D NOESY analysis of **12** indicates 2'-*endo*/3'-*exo* (south) to be the dominant conformation in solution state.

Yet, the correlation of H_2 with both H_1 and H_3 is indeed possible if **12** adopts south conformation (Figure 2), where H_2 and H_3 are aligned in pseudoequatorial axes, while H_1 stands in the pseudoaxial orientation. This claim was additionally supported by the lack of NOE correlation between H_3 and $H_{6\beta}$, as well as H_4 and $H_{6\alpha}$ protons (see supporting information for H_6-α/β assignments). A strong correlation between H_1 and $H_{6\alpha}$ protons further confirmed that south conformation to be the dominant form in

solution state. NOE analysis on a stereochemically well-defined *tert*-butyl group showed strong correlation with H_1 and H_2, an indication of its proximity to these protons, suggesting that these protons are in α configurations. Based on these results, it was safe to conclude that the stereochemical outcomes were in accordance with the expected.

While an S_N2-type condensation of the whole nucleobase was employed in the synthesis of MLN4924 due to issues raised in the indane substitution reaction,[20] substitution reaction worked well in the fluorinated analogue. Reacting **12** with (*S*)-aminoindane in the presence of DIPEA at 90 °C for 48 hours provided **13** in 76% yield. Deprotection of the TBDPS group using TBAF provided the key intermediate **14.**



Scheme 4. Synthesis of target compounds **2** and **3**. Reagents and Conditions: (a) NH_2SO_2Cl, Et_3N, CH_3CN, rt, 16 h, 65%; (b) $TiCl_4$, CH_3CN, 0 °C, 2 h, 60% for **3**, 68% for **2**

The synthesis of final products **2** and **3** was achieved *via* intermediate **14** as shown in Scheme 4. For the preapation of **2**, the sulfamoyl moeity was successfully introduced with freshly prepared sulfamoyl chloride to give the protected fluoro-MLN4924 **15**. Earlier attempts to remove the *tert*-butyl group with acids such as trifluoromethanesulfonic acid proved to be

unsuccessful, however, employing titanium tetrachloride[21] cleanly converted **15** to the target compound **2**. For the preparation of **3**, the same Lewis acid-mediated protocol for the deprotection of the *tert*-butyl group was applied, which successfully furnished target compound **3**. ^{19}F NMR spectroscopy confirmed the presence of fluorine in all final products.

3. Conclusion

In conclusion, we have desiged 2'-β-fluro-MLN4924 **2** and its nucleoside analogue **3** based on bioisosteric rationale and successfully accomplished the asymmetric syntheses of the target compounds by employing stereoselective reduction, regioselective isopropylidene cleavage and DAST fluorination as key steps. Biological significance of these compounds are currently being evaluated and the results will be reported elsewhere. The synthetic approach proposed in this study is expected to serve as a valuable resource in accessing fluorinated carbocyclic nucleosides for further studies.

4. Experimental Section

1-((*tert*-Butyldiphenylsilyl)oxy)-2-((4R,5R)-2,2-dimethyl-5-vinyl-1,3-dioxolan-4-yl)but-3-en-2-ol (4)

i) To a stirred suspension of D-ribose (25 g, 0.17 mol) in acetone (500 mL) at 0 ^{o}C was dropwise added concentrated sulfuric acid (1 mL) under nitrogen atmosphere and the resulting reaction mixture was stirred at room temperature for 2.5 hours. The reaction was quenched by the addition of solid sodium bicarbonate until pH paper indicated solution is neutral. The crude suspension was filtered and concentrated *in vacuo* and the residue was used without any further purification.

ii) The crude residue dissolved in dry dichloromethane (500 mL) was successively added DMAP (47 g, 0.17 mol), triethylamine (47 mL, 0.34

mol) followed by TBDPSCl (44 mL, 0.17 mmol) at 0 ºC under nitrogen atmosphere and the resulting solution was stirred at room temperature for 16 hours. The reaction was quenched by the addition of water (500 mL) and the aqueous layer was extracted with dichloromethane (250 mL x 2). The combined organic layers were washed with brine, dried over anhydrous magnesium sulfate, filtered, and concentrated *in vacuo* and the residue was used without any further purification.

iii) To a stirred suspension of methyltriphenylphosphonium bromide (121 g, 0.34 mol) in dry THF (900 mL) was added potassium *tert*-butoxide (38 g, 0.34 mol) at 0 ºC and the resulting reaction mixture was stirred at room temperature for 1 hour. The reaction was cooled to 0 ºC, then a solution of crude residue, obtained in the previous step, in dry THF (200 mL) was added under nitrogen atmosphere, and the resulting mixture was stirred at room temperature overnight. Upon cooling to 0 ºC, the reaction was quenched with ice water (1 L), and ethyl acetate was added (1 L). The layers were separated and the aqueous layer was extracted with ethyl acetate (500 mL x 3). The combined organic layers were washed with brine, dried over anhydrous magnesium sulfate, filtered, and concentrated *in vacuo*. The crude residue was used without any further purification.

iv) To a stirred solution of oxalyl chloride 2.0 M in dichloromethane (133 mL, 0.27 mol) in dichloromethane (900 mL) was added a solution of DMSO (41.2 mL, 0.58 mol) in dichloromethane (100 mL) at -78 ºC and the reaction mixture was stirred at -78 ºC for 30 minutes. A solution of crude residue obtained from the previous step in dichloromethane (100 mL) was added dropwise and the resulting reaction mixture was stirred for 1 hour. To this mixture was then added triethylamine (141 mL, 1.02 mol) at -78 ºC and the reaction was left to reach room temperature while stirring. After 1 hour, the mixture was cooled to 0 ºC and was quenched carefully with saturated aqueous ammonium chloride solution. The mixture was poured into water (1 L) and the layers were separated. The aqueous layer was extracted with

dichloromethane (500 mL x 2) and the combined organic layers were washed with brine, dried over anhydrous magnesium sulfate, filtered and concentrated *in vacuo*. The crude residue was used without any further purification.

v) To a stirred solution of residue obtained in the previous step in dry tetrahydrofuran (500 mL) was dropwise added vinylmagnesium bromide 1.0 M solution in THF (260 mL, 0.26 mol) at -78 ºC under nitrogen atmosphere and the resulting reaction mixture was stirred at -78 ºC for 1 hour and left to reach room temperature. After stirring for additional 2 hours, the reaction was quenched by slow addition of methanol (300 mL) at 0 ºC and the volatiles were removed *in vacuo*. The residue was poured into water (600 mL) and ethyl acetate was added (300 mL). The layers were separated, and the aqueous layer was extracted with ethyl acetate (300 mL x 2). The combined organic layers were washed with brine, dried over anhydrous magnesium sulfate, filtered and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (hexane : ethyl acetate = 4:1) to afford **4** (43.8 g, 57%), as an inseparable diastereomeric mixture. All spectra was in accordance with the previously reported literature.[17]

(3a*R*,6a*R*)-6-(((*tert*-Butyldiphenylsilyl)oxy)methyl)-2,2-dimethyl-3a,6-a-dihydro-4*H*-cyclopenta[*d*][1,3]dioxol-4-one (5) To a stirred solution of **4** (43.8 g, 96.8 mmol) in dry toluene (1 L) was added Neolyst M2 (4.59 g, 4.84 mmol) at room temperature and the resulting reaction mixture was heated to 60 oC and stirred overnight under nitrogen atmosphere. The reaction mixture was cooled to room temperature, filtered through a pad of Celite and concentrated in vacuo. The crude residue was dissolved in DMF (500 mL) and was successively added 4Å molecular sieves (35 g) and pyridinium dichromate (146 g, 387 mmol), and the resulting reaction mixture was stirred overnight at room temperature under nitrogen atmosphere. The reaction was filtered through a pad of Celite and poured

into water (1 L) and diethyl ether (600 mL). The layers were separated and the aqueous layer was extracted with diethyl ether (400 mL x 3). The combined organic layer was washed with brine, dried over anhydrous magnesium sulfate, filtered and concentrated in vacuo. The crude residue was purified by silica gel column chromatography (hexane:ethyl acetate = 6:1) to afford **5** (29 g, 71%) as a colorless solid. All spectra was in accordance with the previously reported literature.[17] $[\alpha]^{20}_{D}$ = 8.43 (*c* 1.26, CH_2Cl_2); 1H NMR (600 MHz, $CDCl_3$): 7.66-7.63 (m, 4 H), 7.44-7.41 (m, 2H), 7.39-7.36 (m, 4H), 6.32 (t, 1H, *J* = 1.8 Hz), 4.97 (d, 1H, *J* = 5.9 Hz), 4.68 (dd, 1H, *J* = 1.8 Hz, 19 Hz), 4.49-4.47 (m, 2H), 1.34 (s, 3H), 1.32 (s, 3H), 1.08 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$): 201.7, 176.7, 135.4, 132.6, 132.5, 130.0, 127.9, 127.8, 115.4, 78.0, 77.7, 62.4, 27.9, 26.7, 26.2, 19.2; HRMS (FAB+): found 423.1991 [calcd for $C_{25}H_{31}O_4Si]^+$ $(M + H)^+$ 423.1992.

(1*S*,2*S*,3*R*,4*S*)-3-(*tert*-Butoxy)-4-(((*tert*-butyldiphenylsilyl)oxy)methyl) 2-hydroxycyclopentyl pivalate (9) To a stirred solution of **8** (8.01 g, 18.1 mmol) in anhydrous dichloromethane (600 mL) was added 4-dimethylaminopyridine (450 mg, 3.6 mmol), *N*,*N*-diisopropylethylamine (6.3 mL, 36.1 mmol), followed by pivaloyl chloride (2.7 mL, 21.7 mmol) at 0 ºC under nitrogen atmosphere and the resulting solution was stirred at room temperature overnight. The reaction was quenched by the addition of saturated aqueous ammonium chloride (500 mL) and the layers were separated. The aqueous layer was extracted with dichloromethane (300 mL x 2) and the combined organic layer was washed with brine, dried over anhydrous magnesium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (hexane:ethyl acetate = 4:1) to afford **9** (8.37 g, 88%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): 7.89-7.86 (m, 4H), 7.39-7.37 (m, 6H), 4.85 (m, 1H), 4.02-4.00 (m, 2H), 3.74 (m, 1H), 3.59 (m, 1H), 3.32 (d, *J* = 8.0 Hz, 1H), 2.11-2.07 (m,

2H), 1.89 (m, 1H), 1.22 (s, 9H), 1.16 (s, 9H), 1.06 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): 178.3, 135.6, 133.3, 129.6, 127.6, 74.3, 73.2, 71.8, 71.6, 63.2, 41.2, 38.7, 29.8, 28.2, 27.2, 26.8, 19.2; HRMS (FAB+): found 527.3186 [calcd for $C_{31}H_{47}O_5Si]^+$ $(M + H)^+$ 527.3193.

(1*S*,2*R*,3*R*,4*S*)-3-(*tert*-Butoxy)-4-(((*tert*-butyldiphenylsilyl)oxy)methy-l)-2-fluorocyclopentyl pivalate (10) To a stirred solution of **9** (2.72 g, 5.17 mmol) in anhydrous dichloromethane (70 mL) was added pyridine (1.25 mL, 15.5 mmol), followed by DAST (1.36 mL, 10.3 mmol) dropwise at 0 ^oC under nitrogen atmosphere and the resulting reaction mixture was stirred at room temperature for 3 hours. The reaction was quenched with saturated aqueous sodium bicarbonate and the aqueous phase was extracted with dichloromethane and the volatiles were removed in vacuo by co-evaporating with toluene twice. The crude residue was diluted with dichloromethane and successively washed with saturated copper sulfate solution and brine, the combined organic layer was dried over anhydrous magnesium sulfate, filtered and concentrated in vacuo. The crude product was purified by silica gel column chromatography (hexane:ethyl acetate = 10:1) to afford **10** (2.19 g, 80%) as a colorless oil.; ^{1}H NMR (400 MHz, $CDCl_3$): 7.71-7.65 (m, 4H), 7.44-7.34 (m, 6H), 5.01 (m, 1H), 4.95-4.81 (m, 1H), 4.04 (m, 1H), 3.70-3.51 (m, 2H), 2.32-2.19 (m, 2H), 1.69 (m, 1H), 1.19 (s, 9H), 1.14 (s, 9H), 1.04 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): 178.1, 135.7, 135.6, 133.6, 129.6, 127.6, 127.6, 101.3, 99.9, 75.7, 75.5, 74.2, 73.7, 73.5, 62.4, 41.7, 38.6, 30.1, 28.2, 27.1, 26.9, 19.2; HRMS (FAB+): found 529.3138 [calcd for $C_{31}H_{46}FO_4Si]^+$ $(M + H)^+$ 529.3149.

(1*S*,2*R*,3*R*,4*S*)-3-(*tert*-Butoxy)-4-(((*tert*-butyldiphenylsilyl)oxy)methy-l)-2-fluorocyclopentan-1-ol (11) To a stirred solution of **10** (2.04 g, 3.86 mmol) in methanol (50 mL was added sodium methoxide (418 mg, 7.73 mmol) and the reaction mixture was stirred at 50 ^oC for 16 hours. The

reaction was quenched with saturated aqueous ammonium chloride at 0 ºC and the volatiles were removed in vacuo. The crude residue was diluted ethyl acetate and poured into water, and the aqueous layer was extracted with ethyl acetate twice. The combined organic layer was washed with brine, dried over anhydrous magnesium sulfate, filtered and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (hexane:ethyl acetate = 4:1) to afford **11** (1.71 g, 99%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): 7.70-7.66 (m, 4H), 7.44-7.36 (m, 6H), 4.82-4.68 (m, 1H), 4.12-4.09 (m, 2H), 3.73-3.72 (m, 2H), 2.60 (bs, 1H), 2.37-2.16 (m, 2H), 1.51 (m, 1H), 1.18 (s, 9H), 1.05 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): 135.7, 135.6, 135.5, 133.6, 133.4, 129.6, 127.6, 102.9, 101.5, 74.7, 74.5, 74.74.3, 62.7, 53.4, 42.8, 34.4, 28.2, 28.1, 26.9, 26.7, 19.1; HRMS (FAB+): found 445.2568 [calcd for $C_{26}H_{38}FO_3Si]^+$ $(M + H)^+$ 445.2574.

7-((1*R*,2*R*,3*R*,4*S*)-3-(*tert*-Butoxy)-4-(((*tert*-butyldiphenylsilyl)oxy)methyl)-2-fluorocyclopentyl)-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (12)

To a stirred solution of **11** (1.45 g, 3.27 mmol) in anhydrous tetrahydrofuran (60 mL) was added triphenylphosphine (2.14 g, 8.18 mmol) followed by 7-deaza-6-chloropurine (1.00 g, 6.54 mmol) under nitrogen atmosphere and the resulting reaction mixture was cooled to 0 ºC. To this solution was then dropwise added DIAD (1.61 mL, 8.18 mmol) at 0 ºC and the resulting solution was stirred at room temperature overnight. The volatiles were removed *in vacuo* and the crude residue was purified by silica gel column chromatography (hexane:ethyl acetate = 4:1) to afford **12** (1.44 g, 76%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): 8.61 (s, 1H), 7.69-7.67 (m, 4H), 7.45-7.36 (m, 7H), 6.60 (d, J = 3.7 Hz, 1H), 5.66-5.56 (m, 1H), 4.91-4.80 (m, 1H), 4.27 (m, 1H), 3.80 (m, 1H), 3.69 (m, 1H), 2.66 (m, 1H), 2.23-2.12 (m, 2H), 1.16 (s, 9H), 1.05 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): 151.9, 151.5, 150.4, 135.6, 133.7, 133.6, 129.7, 128.8, 127.7, 117.5, 99.5,

98.1, 96.6, 74.6, 74.5, 62.8, 53.6, 53.5, 53.4, 42.4, 30.8, 28.1, 26.9, 19.2; HRMS (FAB+): found 580.2566 [calcd for $C_{32}H_{40}ClFN_3O_2Si]^+$ $(M + H)^+$ 580.2562.

7-((1*R*,2*R*,3*R*,4*S*)-3-(*tert*-Butoxy)-4-(((*tert*-butyldiphenylsilyl)oxy)methyl)-2-fluorocyclopentyl)-*N*-((*R*)-2,3-dihydro-1*H*-inden-1-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (13) To a stirred solution of **12** (1.44 g, 2.48 mmol) in *n*-butanol (20 mL) was added (*S*)-1-aminoindane hydrochloride (2.11 g, 12.4 mmol), followed by diisopropylethylamine (4.33 mL, 24.8 mmol) at room temperature and the resulting reaction mixture was stirred at 90 ºC for 48 hours under nitrogen atmosphere. The reaction was cooled to room temperature and the volatiles were removed *in vacuo*. The crude residue was purified by silica gel column chromatography (hexane:ethyl acetate = 1:1) to afford **13** (1.28 g, 76%) as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$): 8.35 (s, 1H), 7.68-7.66 (m, 4H), 7.45-7.33 (m, 7H), 7.29-7.03 (4H), 6.38 (s, 1H), 5.91 (s, 1H), 5.62-5.52 (m, 1H), 4.90-4.79 (m, 1H), 4.24 (m, 1H), 3.81 (m, 1H), 3.67 (m, 1H), 3.05 (m, 1H), 2.93 (m, 1H), 2.76-2.63 (m, 2H), 2.26-2.08 (m, 2H), 2.05 (s, 1H), 1.13 (s, 2H), 1.18 (s, 9H), 1.07 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$): 156.0, 151.7, 150.4, 143.8, 143.6, 135.6, 133.8, 129.6, 128.0, 127.8, 127.6, 126.8, 124.8, 124.3, 123.7, 98.3, 97.1, 74.6, 74.5, 74.4, 62.9, 53.0, 52.9, 42.5, 34.7, 30.9, 30.2, 28.1, 26.9, 19.2; HRMS (FAB+): found 677.3685 [calcd for $C_{41}H_{50}FN_4O_2Si]^+$ $(M + H)^+$ 677.3687.

((1*S*,2*R*,3*R*,4*R*)-2-(*tert*-Butoxy)-4-(4-(((*S*)-2,3-dihydro-1*H*-inden-1-yl)amino)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)-3-fluorocyclopentyl)methanol (14) To a stirred solution of **13** (1.09 g, 1.61 mmol) in anhydrous THF (50 mL) was added TBAF 1.0 M in THF solution (2.5 mL, 2.5 mmol) dropwise at 0 ºC and the resulting reaction mixture was stirred at room temperature under nitrogen atmosphere overnight. The volatiles were removed *in vacuo*

and the crude residue was purified by silica gel column chromatography (hexane:ethyl acetate = 3:1) to afford **14** (685 mg, 97%) as a brown solid. ^{1}H NMR (500 MHz, $CDCl_3$): 8.39 (s, 1H), 7.35-7.12 (m, 5H), 6.32 (d, *J* = 2.2 Hz, 1H), 5.88 (d, *J* = 7.1 Hz, 1H), 5.59-5.48 (m, 1H), 5.19 (bs, 1H), 4.94-4.83 (m, 1H), 4.37 (m, 1H), 3.90 (m, 1H), 3.72 (m, 1H), 3.06-3.00 (m, 1H), 2.96-2.89 (m, 1H), 2.81 (bs, 1H), 2.76-2.69 (m, 1H), 2.48-2.42 (m, 1H), 2.27-2.20 (m, 1H), 2.00-1.93 (m, 1H), 1.28 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): 158.6, 153.0, 146.0, 145.4, 129.5, 128.4, 126.5, 126.0, 125.4, 125.3, 100.8, 100.2, 99.0, 77.1 ,77.0, 76.7, 62.3, 55.4, 55.3, 44.1 ,35.7, 32.3, 31.8, 29.3; HRMS (FAB+): found 439.2503 [calcd for $C_{25}H_{32}FN_4O_2]^+$ (M + H)$^+$ 439.2509.

((1*S*,2*R*,3*R*,4*R*)-2-(*tert*-Butoxy)-4-(4-(((*S*)-2,3-dihydro-1*H*-inden-1-yl)amino)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)-3-fluorocyclopentyl)methyl sulfamate (15) To a stirred solution of **14** (60 mg, 0.14 mmol) in anhydrous acetonitrile (3 mL) was added triethylamine (0.38 mL, 2.72 mmol) followed by freshly prepared sulfamoyl chloride 2.0 M in acetonitrile solution (0.68 mL, 1.36 mmol) dropwise at 0 ºC under nitrogen atmosphere and the resulting reaction mixture was stirred at room temperature overnight. The reaction was quenched by the addition of methanol, and the volatiles were removed *in vacuo*. The crude residue was purified by silica gel column chromatography (hexane:ethyl acetate = 3:7) to afford **15** (45.5 mg, 65%) as a brown solid. ^{1}H NMR (500 MHz, CD_3OD): 8.20 (s, 1H), 7.26-7.13 (m, 5H), 6.64 (d, J = 3.65 Hz, 1H), 5.85 (t, $J_1 = J_2$ = 7.6 Hz 1H), 5.53-5.47 (m, 1H), 4.93-4.82 (m, 1H), 4.37 (m, 1H), 4.33-4.30 (m, 1H), 4.21-4.17 (m, 1H), 3.78-3.34 (d, 1H), 3.21 (dd, J_1 = 7.35 Hz, J_2 = 15 Hz, 1H), 3.08-3.03 (m, 1H), 2.99-2.88 (m, 2H), 2.3 (m, 1H), 2.35-2.29 (m, 1H), 2.24-2.17 (m, 1H), 2.05-1.98 (m, 1H), 1.28 (s, 9H); ^{13}C NMR (125 MHz, CD_3OD): 158.6, 153.0, 146.0, 145.4, 129.5, 128.4, 126.5, 126.0, 125.4, 125.3, 105.3, 101.0, 100.2, 98.7, 77.1, 76.3, 76.1, 70.8, 62.3, 57.7, 55.4, 55.2, 41.4, 35.7, 32.0,

31.2, 29.2; HRMS (FAB+): found 518.2229 [calcd for $C_{25}H_{33}FN_5O_4S]^+$ (M + H)$^+$ 518.2237.

((1*S*,2*R*,3*R*,4*R*)-4-(4-(((*S*)-2,3-Dihydro-1*H*-inden-1-yl)amino)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)-3-fluoro-2-hydroxycyclopentyl)methyl sulfamate (2) To a stirred solution of **15** (18 mg, 0.034 mmol) in anhydrous acetonitrile (3 mL) was dropwise added titanium tetrachloride 1.0 M solution in dichloromethane (0.52 mL, 0.52 mmol) at 0 ºC under nitrogen atmosphere and the resulting reaction mixture was left to stir for 2 hours at 0 ºC. The reaction was quenched by the addition of water (10 mL) at 0 ºC and the resulting solution was extracted with ethyl acetate (25 mL x 3). The combined organic layer was washed with brine, dried over anhydrous magnesium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography to afford **2** (11 mg, 68%) as a white solid. ^{1}H NMR (400 MHz, CD_3OD): 8.18 (s, 1H), 7.26-7.13 (m, 5H), 6.62 (d, J = 3.6 Hz, 1H), 5.86 (t, J = 9.6 Hz, 1H), 5.66-5.48 (m, 1H), 4.92-4.77 (m, 1H), 4.43-4.37 (m, 2H), 4.27-4.22 (m, 1H), 3.09-3.03 (m, 1H), 3.00-2.85 (m, 2H), 2.67-2.59 (m, 1H), 2.38-2.30 (m, 1H), 2.26-2.18 (m, 1H), 2.06-1.96 (m, 1H); ^{13}C NMR (150 MHz, CD_3OD): 158.7, 150.1, 146.0, 145.4, 129.5, 128.4, 126.5, 126.0, 125.3, 125.3, 105.3, 100.9, 100.1, 98.8, 75.4, 75.2, 71.0, 57.7, 56.0, 41.8, 35.7, 31.9, 31.7; ^{19}F NMR (376 MHz, MeOD): -197.3 to -197.5 (m); HRMS (FAB+): found 462.1603 [calcd for $C_{21}H_{25}FN_5O_4S]^+$ (M + H)$^+$ 462.1611.

(1*R*,2*R*,3*R*,5*S*)-3-(4-(((*S*)-2,3-Dihydro-1*H*-inden-1-yl)amino)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)-2-fluoro-5-(hydroxymethyl)cyclopentan-1-ol (3) To a stirred solution of **14** (8.8 mg, 0.02 mmol) in anhydrous acetonitrile (3 mL) was dropwise added titanium tetrachloride 1.0 M solution in dichloromethane (0.3 mL, 0.3 mmol) at 0 ºC under nitrogen atmosphere and the resulting reaction mixture was left to stir for 2 hours at 0 ºC. The

reaction was quenched by the addition of water (10 mL) at 0 ºC and the resulting solution was extracted with ethyl acetate (25 mL x 3). The combined organic layer was washed with brine, dried over anhydrous magnesium sulfate and concentrated in vacuo. The crude residue was purified by silica gel column chromatography to afford **3** (4.6 mg, 60%) as a white solid. ^{1}H NMR (600 MHz, CD_3OD) 8.18 (s, 1H), 7.25-7.13 (m, 5H), 6.62 (d, *J* = 3.7 Hz, 1H), 5.85 (t, *J* = 7.3 Hz, 1H), 5.59-5.54 (m, 1H), 4.87-4.79 (m, 1H), 4.38 (m, 1H), 3.84 (m, 1H), 3.72 (m, 1H), 3.07-3.02 (m, 1H), 2.94-2.89 (m, 1H), 2.70 (m, 1H), 2.64 (m, 1H), 2.26 (m, 1H), 2.19 (m, 1H), 2.03 (m, 1H); ^{13}C NMR (150 MHz, CD_3OD): 146.0, 145.4, 129.5, 128.4, 126.5, 126.0, 125.4, 100.3, 101.0, 100.3, 99.1, 76.3, 76.1, 63.1, 57.7, 55.8, 55.7, 44.4, 35.7, 31.9; ^{19}F NMR (376 MHz, CD_3OD): -197.2 to -197.4 (m); HRMS (FAB+): found 383.1887 [calcd for $C_{21}H_{24}FN_4O_2]^+$ $(M + H)^+$ 383.1883.

5. References

1. E. Reinstein, A. Ciechanover. *Ann Intern Med.* **2006**, *145*, 676-684.
2. A. Ciechanover. *Cell Death Differ*. **2005**, *12*, 1178-1190.
3. A. Hershko, A. Ciechanover. *Annu. Rev. Biochem*. **1998**, *67*, 425-479.
4. G. Nalepa, M. Rolfe, J. W. Harper. *Nat. Rev. Drug Discov.* **2006**, *5*, 596-613.
5. (a) P. G. Richardson, B. Barlogie, J. Berenson, S. Singhal, S. Jagannath, D. Irwin, S. V. Rajkumar, G. Srkalovic, M. Alsina, R. Alexanian, D. Siegel, R. Z. Orlowski, D. Kuter, S. A. Limentani, S. Lee, T. Hideshima, D.-L. Esseltine, M. Kauffman, J. Adams, D. P. Schenkein, K. C. Anderson. *N. Engl. J. Med.* **2003**, *348*, 2609-2617.; (b) R. C. Kane, R. Dagher, A. Farrell, C.-W. Ko, R. Sridhara, R. Justice, R. Pazdur. *Clin. Cancer Res.* **2007**, *13*, 5291-5294.
6. R. Z. Orlowski, D. J. Kuhn. *Clin. Cancer Res.* **2008**, *14*, 1649-1657.

7. O. Kerscher, R. Felberbaum, M. Hochstrasser. *Annu. Rev. Cell Dev. Biol.* **2006**, *22*, 159-180.
8. Z. Q. Pan, A. Kentsis, D. C. Dias, K. Yamoah, K. Wu. *Oncogene* **2004**, *23*, 1985-1997.
9. T. A. Soucy, P. G. Smith, M. Rolfe. *Clin. Cancer Res.* **2009**, *15*, 3912-3916.
10. R. J. Deshaies. *Nature* **2009**, *458*, 709-710.
11. (a) J. J. Shah, A. J. Jakubowiak, O. A. O'Connor, R. Z. Orlowski, R. D. Harvey, M. R. Smith, D. Lebovic, C. Diefenbach, K. Kelly, Z. Hua, A. J. Berger, G. Mulligan, H. M. Faessel, S. Tirrell, B. J. Dezube, S. Lonial. *Clin. Cancer Res.* **2016**, *22*, 34-43.; (b) S. Bhatia, A. C. Pavlick, P. Boasberg, J. A. Thompson, G. Mulligan, M. D. Pickard, H. Faessel, B. J. Dezube, O. Hamid. *Invest. New Drugs* **2016**, *34*, 439-449.; (c) J. Sarantopoulos, G. I. Shapiro, R. B. Cohen, J. W. Clark, J. S. Kauh, G. J. Weiss, J. M. Cleary, D. Mahalingam, M. D. Pickard, H. M. Faessel, A. J. Berger, K. Burke, G. Mulligan, B. J. Dezube, R. D. Harvey. *Clin. Cacner Res*. **2016**, *22*, 847-857.
12. (a) F. S. Wolenski, C. D. Fisher, T. Sano, S. D. Wyllie, L. A. Cicia, M. J. Gallacher, R. A. Baker, P. J. Kirby, J. J. Senn. *Cell Death Dis.* **2015**, *1*, 15034.; (b) T. A. Soucy, P. G. Smith, M. A. Milhollen, A. J. Berger, J. M. Gavin, S. Adhikari, J. E. Brownell, K. E. Burke, D. P. Cardin, S. Critchley, C. A. Cullis, A. Doucette, J. J. Garnsey, J. L. Gaulin, R. E. Gershman, A. R. Lublinsky, A. McDonald, H. Mizutani, U. Narayanan, E. J. Olhava, S. Peluso, M. Rezaei, M. D. Sintchak, T. Talreja, M. P. Thomas, T. Traore, S. Vyskocil, G. S. Weatherhead, J. Yu, J. Zhang, L. R. Dick, C. F. Claiborne, M. Rolfe, J. B Bolen, S. P. Langston. *Nature* **2009**, *458*, 732-736.
13. N. A. Meanwell. *J. Med. Chem.* **2018**, *61*, 5822-5880.
14. P. Liu, A. Sharon, C. K. Chu. *J. Fluor. Chem.* **2008**, *129*, 743-766.

15. P. Shah, A. D. Westwell. *J. Enzyme Inhib. Med. Chem*. **2007**, *22*, 527-540.
16. P. L. Bonate, L. Arthaud, W. R. Cantrell, Jr., K. Stepehnson, J. A. Secrist III, S. Weitman. *Nat. Rev. Drug Discov*. **2006**, *5*, 855-863.
17. W. J. Choi, H. R. Moon, H. O. Kim, B. N. Yoo, J. A. Lee, D. H. Shin, L. S. Jeong. *J. Org. Chem.* **2004**, *69*, 2634-2636.
18. H. W. Lee, S. K Nam, W. J. Choi, H. O. Kim, L. S. Jeong. *J. Org. Chem.* **2011**, *76*, 3557-3561.
19. W. J. Middleton. *J. Org. Chem*. **1975**, *40*, 574-578.
20. H.-R. Kim, Y, E. Hyun, D. B. Jarhad, J. Yu, L. S. Jeong. *Org. Chem. Front*. **2019**, *6*, 2480-2487.
21. R. H. Schlessinger, R. A. Nugent. *J. Am. Chem. Soc.* **1982**, *104*, 1116.

국문초록

뉴클레오사이드의 당은 용액 상에서 north와 south로 정의되는 두 형태의 빠른 상호전환 상태로 존재한다고 알려져 있다. 당 형태의 고찰을 통해 뉴클레오사이드의 물리화학적 본질에 대한 접근이 이루어졌으며 이러한 과정에서 다양한 합성을 통해 자연에 존재하지 않는 변형 뉴클레오사이드가 탄생했고, 여러 생물학적 활성 평가를 통해 의약품으로서의 가능성을 인정받았다.

형태를 효율적으로 고정시키는 방법으로 bicyclo[3.1.0]hexane 골격이 제안되었다. 이는 삼원환 고리의 위치에 따라 north, 혹은 south 형태로 뉴클레오사이드를 고정시켜 생물학적 활성 평가를 가능케하였으며, 세계 각지의 많은 연구를 통해 무한한 가능성이 제시되고 있다. 하지만, bicyclo[3.1.0]hexane 골격은 합성이 어렵다는 단점을 가지고 있으며 특히 south로 고정된 뉴클레오사이드의 경우 그 합성이 더욱 어려워 향후 의약품으로서의 가치를 가지기 위해 효율적인 합성방법론에 대한 연구가 필요하다. 이에, 본 연구를 통해 bicyclo[3.1.0]hexane 골격을 활용하여 south로 고정된 뉴클레오사이드의 효율적 합성방법론을 개발하고자 하였다. 결과적으로, 쉽고 저렴하게 구할 수 있는 D-ribose로부터 15 step에 걸쳐 총 7% 수율로 south methanocarba uridine의 합성을 완성할 수 있었다. 본 연구 결과는 향후 형태가 고정된 뉴클레오사이드 개발에 큰 기여를 할 것으로 생각된다. 또한, 이를 토대로 당의 형태에 따른 leadzyme의 self-cleaving activity 측정을 하고자 하였으며 이에 south methanocarba cytidine phosphoramidite를 합성하고자 하였다.

NEDD8-Activating enzyme (NAE)의 선택적 저해제로 현재

임상 1상 중에 있는 MLN4924의 효율적인 합성방법론에 대한 연구를 진행하였다. 제시하는 합성방법론으로 regioselective α-alkoxy의 제거와 stereoselective reduction을 핵심 반응으로 사용하였다. 이 방법으로 MLN4924를 15-step에 걸쳐 총 13%의 수율로 MLN4924를 성공적으로 합성하였다. 또한, SAR study를 위해 2번 위치에 불소를 도입한 2'-β-fluoro MLN4924를 설계하였으며 이는 stereselective reduction, regioselective isopropylidene cleavage와 DAST 불소화 반응을 핵심반응으로 하여 성공적으로 합성할 수 있었다.

Keywords: 카보사이클릭 뉴클레오사이드, 형태가 고정된 뉴클레오사이드, MLN4924, 불소화 뉴클레오사이드, 생동등체

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